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Page 12, line 13, *read* (1, p. 33, 37) *for* (1, p. 60); line 31, *insert* under "Cultural Characters": All the beef media used was made with *beef infusion* as a base, unless otherwise stated.

Page 21, *delete* reference 1 and *read* Rapp, C. W. Bacterial blight of beans. Okla. Agr. Exp. Sta. Bul. 131: 2–39. 1920.

Page 178, line 5 from bottom, *read* alsike clover *for* white clover.

Page 341, line 7 from bottom, *read* perithecia *for* pirithecia.

Page 385, line 2, *read* unsterilized *for* sterilized.

Page 423, footnote 2, line 2, *read* 1925 *for* 1924.

Page 518, legend for Fig. 2, line 2, *read* × 1000 *for* × 800.

Page 657, line 33, *read* teratological *for* teretological.

Cover of October number, *read* Albert S. Muller *for* Albert S. Miller.

Page 891, Table 1, column 3, *read* Cavangerie *for* Kavangerie.

Page 990, line 1, *read* it caused *for* is caused.

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BACTERIAL WILT OF BEANS (BACTERIUM FLACCUMFACIENS HEDGES), INCLUDING COMPARISONS WITH BACTERIUM PHASEOLI

FLORENCE HEDGES

INTRODUCTION

The disease described in this paper has been under observation by the writer since 1921, at which time navy bean plants were received from South Dakota accompanied by the statement that large numbers of the plants were dying. The wilted plants were found on the farm of the office of Forage Crop Investigations at Redfield, S. D., in navy beans which had been inoculated with *Bacillus radicicola,* and it was first thought by Mr. Leonard, of the office of Soil Bacteriology Investigations, that the nodule organism might have become pathogenic. This proved not to be the case. It was learned that the previous year there had been a loss of 90 per cent of the crop from what appeared to be the same disease, and that the 1921 crop was grown from seed harvested from the remaining 10 per cent. The loss in 1921 amounted to about 25 per cent. Some of the same seed planted at Arlington, Va., produced a large number of plants that wilted and died in the seedling stage. Examination of the plants from both South Dakota and Virginia showed bacteria in the vessels of the stem, often accompanied by browning. From diseased plants from both sources a yellow organism was isolated, with which the disease was repeatedly produced by artificial inoculation. This organism was found to differ from all bacteria hitherto described as infectious to bean, and was named *Bacterium flaccumfaciens.* A preliminary paper was published in Science (2), and a paper was given before the American Association for the Advancement of Science (3).

GEOGRAPHICAL DISTRIBUTION

The disease has been isolated from wilted navy bean plants from South Dakota, Michigan, Virginia and Maryland; and from seed from South

Dakota, Montana, Michigan, Maryland, District of Columbia, France and Germany. The author believes the disease is rather widely distributed in bean-growing sections but that it has been confused with the blight caused by *Bact. phaseoli*, and that in all probability the greater part, if not all, of the seedling wilt reported by the Plant Disease Survey of the Bureau of Plant Industry in Indiana, Michigan, Minnesota, New Jersey and Maryland in 1917, Minnesota and Connecticut in 1918, and New Jersey in 1920 was due to *Bact. flaccumfaciens*. In the summer of 1922 some Michigan fields showed as high as 90 per cent of the crop affected. It was the most serious disease affecting Michigan beans that season.

DESCRIPTION OF THE DISEASE

The following description applies to the wilt chiefly as it appears on the navy bean, inasmuch as this is the variety on which it has been most commonly observed and principally studied up to the present time.

The disease attacks all parts of the plant above ground. It may either kill the plant in the seedling stage, or dwarf it, resulting in a reduction in yield and the production of diseased seed.

Seedling wilt. Plants not more than 2 or 3 inches high may be attacked. The wilting and shrivelling of the leaves is sometimes accompanied by a dull green or brownish green discoloration, and the whole plant may be dead before it has developed more than the first pair of leaves.

Wilt of older plants. If the plant survives the seedling stage or becomes infected at a later period in its development, it may live throughout the season and mature its seed. When conditions are favorable, the diseased plants are very conspicuous because of the large number of shrivelled leaves (Fig. 1); but under conditions less conducive to severe attack they may be scarcely noted, as the wilting takes place slowly. Sometimes a seemingly vigorous plant bears one or more shrivelled lateral shoots more or less hidden by the mass of healthy foliage. Growers should be on the lookout for these less conspicuous signs, for such plants give the farmer little or no warning and yet produce diseased seed.

The discoloration accompanying the early stages of wilt when the leaves are becoming flabby is not, as a rule, very conspicuous. The color of the affected portion is dull green or brownish green, greenish brown, or reddish brown. The discolored area is flabby at first and later dry and papery. Now and then it is bordered by yellow, but this is not common. Sometimes the whole leaf blade and petiole become flabby and droop, unaccompanied by discoloration of any kind. Usually the wilting of a portion of the leaf is rapidly followed by the collapse of the whole, but occasionally the infection is restricted to a comparatively small area, the rest of the blade and the petiole remaining turgid at least for a time.

Another phase of the disease is a breaking over of diseased plants. In all cases observed, the break has occurred at a node which has been browned or reddened. The occurrence of this phase is probably dependent upon the amount and severity of wind and the degree of infection in the stem. It has been observed in the field only, never in greenhouse experiments even when the inoculation was made in a node. This phenomenon has been reported by Muncie (6) in connection with bacterial blight (*Bact. phaseoli*). From such broken over beans in Michigan in 1923, *Bact. flaccumfaciens* was isolated and its pathogenicity proved by inoculation experiments.

Pod infection. This disease is very much less conspicuous on the pods than bacterial blight (*Bact. phaseoli*). In fact sometimes, even on pods in which all the seeds are infected, there is no outward sign at all (Plate I, A and B) and on others it is so slight as to be easily overlooked. It does not consist of spotting as in blight, but infection takes place by way of the fibro-vascular bundles in the pedicel and manifests itself as a discoloration running along one or both sutures and sometimes spreading laterally to some extent. Infection may be observed on pods which are still green or just yellowing. On green pods the diseased area may be a rather sickly green or a yellowish green, or it may be somewhat darker than the rest of the pod and more or less watery looking. On ripe pods it is more conspicuous, the olive greenish color of the infected suture being then in greater contrast to the yellow color of the normal portion of the pod (Plate I, C). Infected pods have been found on large vigorous plants showing no other sign of the disease.

Seed infection. Diseased plants bear infected seed, but observations made thus far indicate that only a small proportion are visibly infected. There are two types of seed infection: (I) systemic or internal (Plate I, A), and (II) superficial. When seeds are harvested from an infected field, bright yellow seeds are very conspicuous among them. The infection is of type I and easily seen by the workers who hand-pick the seed in commercial seed houses. The culls from Michigan elevators in 1922 and 1923 contained large numbers of infected seeds and yet a sufficient number are overlooked, as shown by an examination of many packages of commercial seeds, to start the wilt in the fields the following year. The yellow color is caused by a thick bright yellow bacterial layer underneath the white seed-coat and visible through it. If the seed-coat is removed, this layer can be scraped up with the point of a knife, leaving the seed-coat white. Sufficient bacteria are present in a badly infected seed to make 10 cc. of water very milky. Sometimes the whole seed is yellow, sometimes only a portion, and sometimes it has a speckled appearance. Often such seeds are shrivelled, but they are quite as often plump and of normal size.

At times the infection is purely superficial (type II), bacterial slime being smeared on the surface of the seed from the interior of the pod, and sometimes the only sign of infection is a small amount of yellow slime at the hilum. Such seeds are sure to be overlooked by the farmer who saves his own seed or by the hand-pickers in the commercial seed houses. Unfortunately, the germination of even heavily infected seed does not seem to be greatly impaired, tests showing a germination of 72 per cent.

These tests were made with yellow seeds selected from culls from a Michigan elevator, so they were presumably from many different lots of seed and no checks were available.

Both types of seed infection may occur in cases of bacterial blight (*Bact. phaseoli*) also, and when they do occur can not be differentiated by the eye alone from those due to bacterial wilt (*Bact. flaccumfaciens*).

The writer believes, however, that type I (systemic or internal infection) is rare in cases of bacterial blight (*Bact. phaseoli*), because systemic infection of the plant as a whole is rare in that disease, though it does occur. Furthermore, there is the circumstantial evidence that but three isolations from 18 samples of diseased seed (type I), from as many sources, yielded the blight organism (*Bact. phaseoli*), whereas the other fifteen platings gave pure cultures of *Bact. flaccumfaciens,* the wilt organism, regardless of the fact that the bacterial blight (*Bact. phaseoli*) occurs very commonly in all bean-growing sections.

The writer is of the opinion, from observations made up to the present time, that the greater part of the yellow seed answering the above description is infected with the wilt (*Bact. flaccumfaciens*) instead of the blight organism (*Bact. phaseoli*) and that these two diseases have been confused on the seed as well as in the field.

HOST PLANTS

The disease has been found occurring naturally on navy, wax and lima beans and has been produced artificially in soy beans (Var. Ito San). Tests of other legumes and related plants are contemplated.

PLANTING OF DISEASED SEED

On May 20, 1922, seed from the diseased crops grown in 1920 and 1921 at Redfield, S. Dak., was planted at Tuxedo, Md., on land on which beans had never before been grown. Yellow seed from two other lots, bought on the market in Baltimore and Washington, and known to contain *Bact. flaccumfaciens,* also were planted in this plot, altogether about 28½ oz. of seed. Unfortunately, the writer was unable to obtain, that season, any commercial samples of navy bean seed wholly free from wilt and accordingly was

obliged to use for the check plot seed selected with as much care as possible from lots known to be infected.

Two weeks after planting, when the plants were about 2 inches high, most of them had developed the first compound leaf and there was 2.2 per cent wilt. No wilt had developed in the check plot.

Examinations were made of the plot from time to time during the summer, and an increased number of wilted plants was found on each successive examination.

The results of the experiment were inconclusive because of the impossibility of obtaining good seed for checks and because the plants in two whole rows, including the checks, became badly infected with mosaic. Considerable blight (*Bact. phaseoli*) also was present.

On August 28 the crop was harvested. The plants were much stunted and had produced very few pods. Of the 15 per cent which still bore living shoots, 88 per cent were examined microscopically and 70 per cent were found to contain bacteria in the stem. The plants bore very few pods, and many of these were infected with *Bact. flaccumfaciens*. Many cases of wilt occurred among the checks also, a result which was not surprising. The experiment would not have been worth recording had it not been that no beans had ever been grown on this ground before and there were none in the vicinity from which the disease might have been carried.

Wilt also occurred on plants grown from diseased seed in sterilized soil in the greenhouse (Plate II, E).

FIELD OBSERVATIONS

Arlington, Va. The writer first saw this disease in the field at the Arlington Experiment Farm, Va., Aug. 30, 1921, in an experimental plot belonging to Mr. Leonard, of the office of Soil Bacteriology Investigations. Mr. Leonard had undertaken to determine what influence, if any, the treatment of bean seed incidental to its inoculation with liquid cultures of *Bacillus radicicola* might have upon bean wilt (5), that disease having been first observed in conjunction with inoculation with the root nodule organism at Redfield, S. D. Seed from the diseased Redfield crop and from the markets in Baltimore and Washington, D. C., was used. Wilt occurred in plants from all three lots, but the writer's observations were concerned chiefly with the progeny of the Redfield seed.

The plants were in the seedling stage, and great numbers of them had wilted and died. The beans were planted in hills, and in some cases as many as five plants in seven were wilted. Ten partially wilted plants were taken up and planted in pots in the greenhouse. For a time they continued to grow slowly, the new leaves wilting shortly after unfolding, with the

exception of one plant more resistant than the rest. In 5 days, 5 of the seedlings had no turgid leaves and 5 had one each. In 12 days, 5 were dead; and in 6 weeks, 7 of the 10 were dead and 2 nearly so. The remaining plant had been much more resistant. It had grown more rapidly than the others and, whereas, on the 5th day it had only one turgid leaf, on the 12th day it had 3 and on the 22nd day it still had two which showed no sign of wilt. At the end of 6 weeks the last leaf of this most resistant plant of the ten was flabby, and the plant finally succumbed to the disease.

A final examination was made of the Arlington plot on Oct. 10. The majority of the plants were very much stunted, the leaves were abnormally small and many of them were flabby or dead. The tips of many small lateral shoots were wilted and shrivelled. Many plants were entirely dead and few appeared to be normal.

Michigan. In the summer of 1922, Dr. R. D. Rands, of the Bureau of Plant Industry, made an extended survey of the bean-growing districts in Michigan and reported the occurrence of much wilt there, stating that it was the most serious bean disease in the state that season. Infected plants collected by him were turned over to the writer, who isolated very virulent strains of *Bact. flaccumfaciens* from the seeds. The following summer (1923) the writer visited several of the largest bean-growing centers in the state during the latter part of June and of August. Because of the prevalence of the wilt the preceding year, it seemed probable that a considerable amount of infected seed would find its way into the fields at planting time and that seedling wilt would result. Such proved to be the case, although only one field was found where the wilt was present in quantity at the time of the first visit in June. This was a 10-acre field of Robust bean and it was badly infected with seedling wilt, although no dead plants were observed, and most of the plants had only one or two wilting leaves. An examination of the seed left after the planting of this field showed a considerable proportion of infected seed.

On this tour of inspection infection was found on both Robust and Improved Robust, two varieties of navy bean widely grown and highly prized in Michigan because of their resistance to disease. Early Wonder also was found to be attacked. In several instances where wilt occurred the writer had an opportunity to examine the seed left from the planting, and in every case it was found to be infected. Infected seed was very common in a sample examined at one of the elevators in Tuscola County, the county in which the greatest amount of wilt was found.

In August the same fields were re-visited, and wherever seedling wilt had been observed in June, infected seed was found in August. Infected pods and seed were very plentiful in the field previously mentioned, in

which so much seedling wilt was seen in the spring, although, owing to the excessively dry weather, no doubt, it had been so inconspicuous during the summer that the owner of the field had believed his plants had outgrown the disease and was much amazed when the infected pods were pointed out to him. He had been greatly interested in the writer's photographs and colored plates of the wilt on the previous visit and had been on the lookout

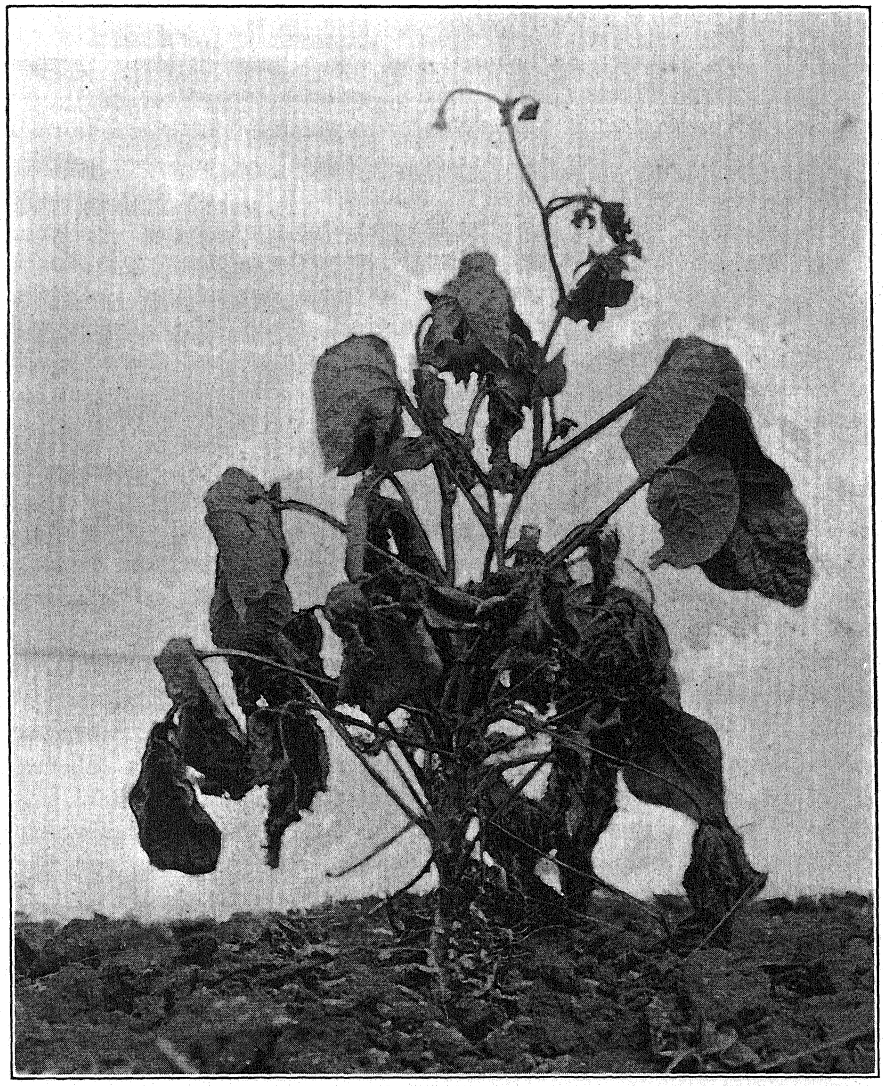

FIG. 1.—Wilted navy bean plant from badly infested field, Redfield, S. D. Courtesy of L. T. Leonard, U. S. Dept. of Agr.

for the disease during the summer. This is an instance of the manner in which, under certain conditions, the wilt can persist in a field and produce diseased seed without attracting notice. Although there had been much seedling wilt in the spring, the disease had progressed so slowly during the dry season that few plants had wholly succumbed, and the wilted leaves had been so few at any one time and dropped off so gradually that the field appeared to be free from the disease. This was in marked contrast to the Redfield, S. D., field in 1920 and 1921 and fields in Michigan the previous year (1922) as reported by Leonard and Rands, respectively. In these fields badly wilted plants (fig. 1) were of common occurrence.

In one Michigan field of Early Wonder in which but little seedling wilt was observed in June, there was a great quantity of pod and seed infection (Plate I, C). This field also had been planted with infected seed, as shown by examination of the seed left after the planting.

The breaking over of the stems near the ground was observed in August in one of the experimental plots at the Michigan Agricultural College in which the plants were badly wilted. Nearly all of the plants affected with wilt were broken in this manner. From such plants, *Bact. flaccumfaciens* was isolated. The breaking was usually accompanied by browning or reddening of the stem.

ISOLATION OF THE ORGANISM

The organism has been isolated in pure cultures repeatedly from stems, petioles, and leaf blades of both seedlings and older plants, and from the seed. The colonies appeared, as a rule, in two days. When isolations were made from stems, petioles or leaf blades, the tissue was dipped in alcohol, sterilized in mercuric chloride 1:1,000 for twenty seconds to two minutes. Leaf blades were never sterilized more than one minute. The mercuric chloride was then washed off in sterile water, the tissue transferred to beef bouillon or another tube of sterile water and crushed. From the tube containing the crushed tissue, dilutions were made and plates poured.

The organism is easily obtained from the seed in pure cultures without sterilization, both from the bacterial layer under the seed coat and from dried bacterial ooze on the surface. In the case of the former the seed was split, the seedcoat with its bacterial lining removed, dropped into sterile water and left standing a few minutes before the dilutions were made, in order that the bacteria might diffuse. When pouring plates from dried bacterial ooze on the surface of the seed, the whole seed was dropped into sterile water.

The organism is most surprisingly resistant to drying, as is evidenced by the fact that it has been isolated from dried bacterial ooze on the hilum

of seed nearly 5 years old. Even in this case the colonies appeared in 2 days and were just as virulent as isolations from the most recent infections.

INOCULATION EXPERIMENTS

Prick inoculations. Artificial infection has been repeatedly produced by pricking bacterial slime from pure cultures of *Bact. flaccumfaciens* into stems, petioles, or pods of sound plants. Sometimes the plants were put into inoculation cages but this is not necessary. The plants were, however, kept shaded for the first 12 or 24 hours after inoculation. Plants of all ages were inoculated in the stem—from tiny seedlings in which the cotyledons had not yet spread apart to those which were in blossom. The plants are susceptible at all ages if thrifty and growing well. Older plants were usually inoculated near the tip of the main stem or on a lateral shoot, although infection also can be obtained by inoculation near the base of the plant. The first signs of infection usually were observed in 5 to 10 days, although in one experiment wilting occurred in 3 days and in another the first signs of infection were observed in 2 weeks. In one set of inoculations there was 100 per cent infection in 11 days.

The wilting and shrivelling of the first leaf above the pricks was followed by the successive wilting and falling of the leaves above until the stem was defoliated or nearly so (Plate II, A–C). Sometimes the plants were completely defoliated in 14–18 days. At other times the disease progressed much more slowly. If the plants lived long enough to produce pods, these were commonly infected (Plate II, A).

In some of the artificial infections, yellowing occurred. This was quite common in two of the inoculation experiments, one made Dec. 20, 1921, on very young King of the Garden Lima beans and Ito San soy bean seedlings, the other made Oct. 24, 1922, on navy beans 1–2 feet high. Pure cultures of *Bact. flaccumfaciens* were repeatedly obtained by plating from these yellow areas, which appeared in 13–39 days. This phenomenon, however, has been observed too rarely to be considered one of the characteristics of the disease.

Another very successful method of obtaining infection on very young plants was by removing the cotyledons and painting the wound with a brush dipped in a water suspension of *Bact. flaccumfaciens.*

A simple time-saving device was constructed for use when large numbers of plants were to be inoculated, consisting of a small camel's hair brush to which a fine needle had been bound. The point of the needle was about 2 mm. below the end of the brush. With this device the wounding and application of the bacteria were effected at the same time. Since the instrument could not be flamed, as many of them were used as there were colonies concerned in the inoculation.

Very interesting and unexpected results were obtained when prick inoculations were made on the green pods. The Lazy Wife pole bean was the variety used in every case and inoculations were also made with *Bact. phaseoli* for comparison. There was so little reaction on the surface of the pods inoculated with *Bact. flaccumfaciens* in 10–12 days that the writer thought no infection had taken place. In 14–16 days, however, the pods were withering and in some cases yellowing. At this stage they were opened and the seeds were found to be badly infected (Plate III, A). Where the needle pricks had gone into the seeds, there was a bright yellow bacterial layer under the seedcoat. There was also considerable bacterial slime on the inner surface of the pod and in one case the latter was discolored. The interior of the pods inoculated with *Bact. phaseoli* presented an appearance very similar to that just described, but the exterior showed a decided contrast, the pricks in the inoculations with *Bact. phaseoli* being surrounded by water soaking, which appeared in 7 days and left no doubt as to the success of the inoculation.

Prick inoculations also were made on the pedicels and on the stem just below the pedicel. In 3 weeks, infection was visible along the seed-bearing suture, which was darker green than the rest of the pod and in some cases was splitting. There was also bacterial ooze in some instances. In 4 weeks the pods were beginning to wither and were turning a yellowish green in the flabby portion (Plate III, B). The pods were opened and found to contain infected seed. There was conspicuous watersoaking in the pods similarly inoculated with *Bact. phaseoli,* a marked contrast to the preceding. The infection had likewise entered the seeds, which resembled those infected with *Bact. flaccumfaciens.*

All attempts made to infect flowers failed, as in every case they fell off. When extremely young pods were inoculated, they either fell off or failed to mature.

Applying a bacterial suspension of *Bact. flaccumfaciens* to the uninjured surface of green pods with a brush produced no infection, but a similar treatment with *Bact. phaseoli* produced typical watersoaked spots.

Spray inoculations. None of the writer's experiments have given conclusive evidence of the occurrence of stomatal infections and it is the opinion of the author that they do not take place. They certainly do not do so readily, as is the case with *Bact. phaseoli.* Spray inoculations have been made on seedlings of navy beans, King of the Garden Lima and Ito San soy bean, with prick inoculations as checks. The plants were kept in a moist atmosphere at least 24 hours. In one set of these experiments a method was used which had proved very effective in producing infection in soy beans with *Bact. phaseoli,* a difficult accomplishment, *viz.,* spraying

seedlings a few days old in the damp chamber in which they had been germinated (4). Three days later the sprayed seedlings were planted in pots in the greenhouse. The prick inoculations resulted in infection in 5 to 13 days, but whenever infection occurred on the sprayed plants it was on a torn leaf or on a plant which had been so gnawed by insects that it seemed probable that the bacteria had entered through the wounds. In some instances it was very evident that this was the case. No small spots, gradually increasing in size indicative of stomatal infections, were ever observed.

On the other hand, that the bacteria easily can find their way into wounds without being forced in mechanically as in the case of the needle-prick inoculations described above, is evident from the fact that infection readily took place on King of the Garden Lima beans when the plants were pricked first and then sprayed with a water suspension of the bacteria. The plants were kept moist in inoculating cages for 46 hours. Local infection occurred on Ito San soy bean leaves which had been rubbed between the thumb and forefinger after spraying, but no wilt resulted. The infection was confined to brown streaks on the leaves from which *Bact. flaccumfaciens* was re-isolated. On the other hand, this plant succumbs readily to prick inoculations.

Infection was obtained upon all the varieties tested, *viz.*: Great Northern Navy, Common Navy, King of the Mountain Navy, Black Valentine, Lady Washington, Early Wonder, Red Kidney, Wardwell's Kidney Wax, Davis White Wax, Vaughan's Stringless Wax, Lazy Wife, King of the Garden Lima and Ito San soy bean.

The colonies used for the inoculations came from widely separated sources: South Dakota, Montana, Michigan, Virginia, Maryland, France and Germany. All were equally virulent.

SUSCEPTIBLE VARIETIES

The disease has been found occurring naturally on the following varieties: Common Navy, Early Navy, Great Northern Navy, Improved Navy, Navy Pea Bean, Pea Bean, Pilot Navy, Black Valentine, Crystal White Wax, Early Wonder, Golden Eye Wax, Henderson's Bush Lima, H. trés nain précoce, Hinrichs Riesen, Improved Robust, Robust, S.P.I. 50892, S.P.I. 50894, Wardwell's Kidney Wax, White Kidney, and a pink seeded variety from Michigan.

Wardwell's Kidney Wax was reported as being especially susceptible to wilt in Michigan in 1922.

In addition to the above, the following were found by inoculation to be susceptible: Davis White Wax, King of the Garden Lima, King of the

Mountain Navy, Lady Washington, Lazy Wife, Red Kidney, Vaughan's Monster Stringless Wax and Ito San soy bean.

Variety tests on a large scale have been carried out with the wilt organism by Rands and Brotherton (7) of the Bureau of Plant Industry in connection with parallel experiments on mosaic, anthracnose and bacterial blight (*Bact. phaseoli*), with a view to developing varieties resistant to these enemies of the bean crop. No variety wholly immune to *Bact. flaccumfaciens*, however, has been found.

CONTROL METHODS

The use of clean seed from wilt-free fields is the best means of control thus far known, both observation and experiment having shown that this disease is transmitted through the seed. The use of seed two or three years old as recommended by Rapp (1, p. 60) of the Oklahoma Agricultural Experiment Station, as a control for *Bact. phaseoli* would avail nothing in this case, as the causal organism of wilt has been found alive and virulent on seed five years old. It is doubtful whether any form of seed treatment would be practicable, as the bacteria are underneath the seed coat and protected by it. The ease with which the seed coats slip would preclude any long treatment with liquids, and the thermal death point of *Bact. flaccumfaciens* is so high (60° C.) that a temperature which would kill it would probably kill the embryo of the seed. However, experiments along this line are underway.

Until more is known about the possibility of infection through the soil, it is advisable not to use bean straw as fertilizer or feed. Experiments on soil infection have been undertaken.

Leonard (5) states that in his experiments in South Dakota there were indications of a stimulation of the wilt disease when bean seed was inoculated with the root-nodule organism by means of liquid cultures. He suggests the applications of *Bact. radicicola* in the form of inoculated soil applied in a dry condition.

CULTURAL CHARACTERS

Colonies on 1 per cent peptone beef agar plates.—Appear usually in 2 days, whitish at first but very soon becoming pale yellow or Ridgway's cream color (8). They are round, smooth, wet-shining, semi-opaque, with entire, slightly thinner margins, flat or slightly convex, coalesce readily, and are finely granular. On very thinly sown plates they may become 4–7 mm. in diameter. A small brighter yellow type of colony occasionally appears on the plates but this appears to differ in no other respect from the pale

yellow or cream colored type either on other culture media or in its host reactions. No internal markings.

Beef peptone agar slants.—Moderate flat spreading, pale yellow growth in two days at 22° C.: glistening, slightly contoured, translucent and viscid. It is sometimes difficult to remove the slime from the agar and difficult to make it diffuse in water.

Beef peptone agar stabs.—Good pale yellow to deep yellow nailhead covering the surface of the agar. Scanty growth in the stab, very rarely any below 1 cm. in depth.

Beef peptone gelatin stabs.—Very slow liquefaction. Little or no liquid gelatin present during the first month, evaporation taking place almost as rapidly as the liquefaction in contrast to the rapid liquefaction by *Bact. phaseoli.* In 9 weeks the gelatin was 1/3 to 1/2 liquefied.

Congo Red agar (Kellerman).[1]—Very thin, scanty colony-wise growth, colorless at first but later absorbing the Congo red. The agar becomes purplish in 2–4 weeks. An excellent medium for distinguishing *Bact. flaccumfaciens* from *Bact. phaseoli.* The latter makes a copious smooth wet-shining growth in decided contrast to the meager growth of the former.

Lima bean agar.—Very scanty growth, thin, slightly rough, pale yellow.

Litmus lima bean agar.—A very scanty growth bluing the litmus.

String bean agar.—Thin, dull, finely roughened growth, colorless at first but becoming yellowish. An excellent medium for differentiating this organism from *Bact. phaseoli* which makes a good wet-shining growth upon it.

Soil agar (Löhnis).[2]—Very scanty, barely visible growth.

Starch agar.—Very weak diastasic action on cornstarch beef infusion agar. Plates tested with iodine when 1 week old. An excellent means of differentiating *Bact. flaccumfaciens* from *Bact. phaseoli* (Fig. 2).

[1] Distilled H_2O 1,000.00 c.c.
Saccharose 10.00 g.
Dipotassium phosphate 1.00 g.
Magnesium sulphate 0.20 g.
Agar flour 15.00 g.
Congo red 0.10 g.
Steam water, agar and salts one-half hour then add Congo red.

[2] Soil 150 g.
Distilled H_2O 1,000 c.c.
Calcium oxide trace
Heat in steamer 1 hour, filter and add per 1,000 c.c.:
Dipotassium phosphate 0.5 g.
Mannite 10.0 g.
Agar flour 15.0 g.
$CaCO_3$ excess.

Thaxter's potato agar.—Good but not copious, whitish growth, somewhat contoured but for the most part smooth. Dry shining. Excellent medium for differentiating this organism from *Bact. phaseoli,* which makes thereon a copious wet-shining yellow growth.

Whey agar.—Good but not copious growth, dry-shining and somewhat contoured. When 10 days old the bacterial layer varied from primuline yellow to buff or apricot yellow (8). A marked contrast to the copious smooth wet-shining growth of *Bact. phaseoli* on this medium.

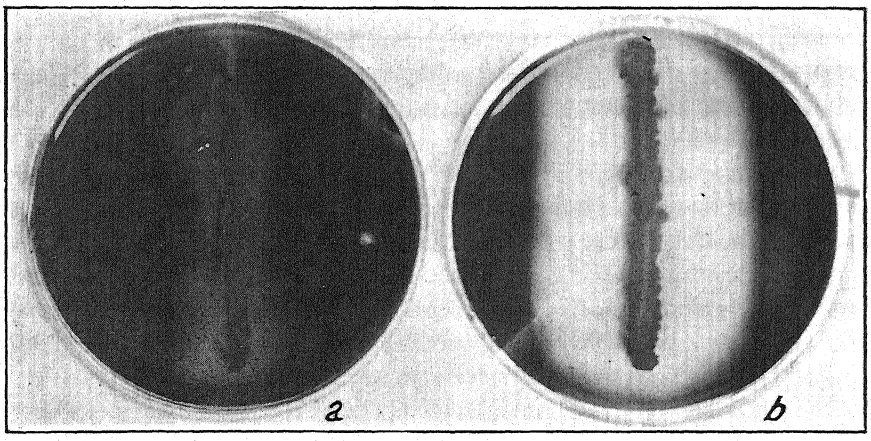

FIG. 2.—Cornstarch beef infusion agar plates 7 days old tested with iodine, showing comparative amount of diastasic action. × ½ ca.

A. *Bact. flaccumfaciens.* Weak diastasic action.

B. *Bact. phaseoli.* Strong diastasic action.

Yeast agar + 1 per cent dextrose.[3]—A very good medium for this organism. There is a thin, whitish colony-wise growth in 24 hours if transferred from young cultures. Excellent whitish or cream-colored, wet-shining, somewhat contoured growth in 3 to 6 days. Color deepens to primuline yellow (8), in old cultures (4–5 weeks) and the bacterial layer becomes smooth.

Potato.—Moderate, often scanty mustard or primuline yellow (8) growth usually accompanied by a marked graying of the potato; very little diastasic action; a striking contrast to the copious growth and strong diastasic action of *Bact. phaseoli.* Growth on potato is extremely variable, sometimes ranging from little or none to very good growth in the same series

[3] 200 grams yeast (Red Star yeast, free from starch is recommended), 2,000 c.c. tap water. Mix thoroughly and heat in steamer for 2–3 hours. Stopper with cotton and autoclave for at least ½ hour. Let stand several days. Siphon off the top liquid, adjust reaction, add agar and sugar.

of cultures of the same colony, probably due to differences in the individual potatoes.

Beef infusion peptone bouillon.—Thin to moderate clouding within 24 hours, often noticeably more clouded at the top, at temperatures ranging from 20° to 36° C. The clouding may increase somewhat and is persistent at favorable temperatures. A partial or complete pellicle usually forms in 2 or 3 days. The character of this varies considerably. When young it shakes down easily, either falling as a whole, breaking up into coarse flocculence or falling in long strings when agitated slightly. In cultures two weeks old which have not been disturbed the pellicle may be thick, exceedingly viscid and difficult to shake down. Sometimes a heavy rim is formed, but many cultures form only a faint partial one. The formation of pseudozoogloea is also an inconstant character. When present, they are sometimes more numerous in the upper portion of the medium.

A very viscid ropy precipitate forms, rising in swirls when shaken and sometimes detached from the bottom of the tube only with the most vigorous shaking. No odor.

Cohn's solution.—Little, if any, growth.

Fermi's solution.—Some colonies cloud this medium very faintly, others not at all. Clouding when it does occur is transient.

Litmus milk.—A slight alkalinity often occurs the first few days. Litmus reduced in 4–12 days. Acid reaction in 3–4 weeks. Other changes as in milk *q. v.*

Milk.—A solid acid curd is formed in milk, although cultures are often slightly alkaline the first few days (compare lactose agar). Coagulation normally begins in 3 or 4 weeks, occasionally in 2 weeks, and on the other hand, sometimes in a series of cultures one which has remained fluid long after the others, will coagulate ultimately.

One to several days after the formation of the solid curd there is an extrusion of whey and shortly thereafter peptonization begins, progressing very slowly. Rare cases have been observed in which peptonization began in 2 weeks without the forming of a solid curd. Tyrosin crystals have been observed in cultures 3 months old. *Bact. flaccumfaciens* usually produces a wide deep, primuline yellow rim in milk which is very striking.

This is a good medium for differentiating this organism from *Bact. phaseoli.*

Uschinsky's solution.—Thin to moderate clouding. No rim, pellicle or pseudozoogloeae.

FERMENTATION

Fermentation tubes.—No gas was produced in fermentation tubes containing beef extract broth with brom cresol purple, 1 per cent peptone and

1 per cent dextrose, lactose and saccharose. Little, if any, clouding in the closed end. Acid reaction in all, visible in saccharose in 18 to 42 hours, in dextrose in 42 hours, and in lactose in 5 to 7 days. Temperature 21–26° C. Alkali was sometimes produced in lactose following the production of acid. In dextrose and saccharose the reaction was still acid after 2 months.

Litmus lactose agar (1 per cent beef infusion agar + 1 per cent peptone + 1 per cent lactose).—Pinkish cream colored growth. Trace of reddening (10 days). Reduction of the litmus. Good medium for differentiating *Bact. flaccumfaciens* from *Bact. phaseoli* when cultures are young. Old cultures (2 mos.) of both organisms are bluish green.

Sugar and glycerine agars with brom cresol purple and phenol red (slant beef extract agar + 1 per cent peptone and 1 per cent of the substance to be tested). There was good growth in all. No gas was produced. *Bact. flaccumfaciens* was yellow on the media containing brom cresol purple but nearly white on that containing phenol red. The cultures were kept at room temperatures which varied considerably during the course of the experiments (21° C. to 32° C.). The results were as follows:

Dextrose.—Acid produced in 2 days. Cultures still acid after 2 months with the exception of one colony which began to show an alkaline reaction in 17 days.

Lactose.—Slight but distinct alkaline reaction in 2 days in most cases. Acid produced in 3–5 days. Occasionally a colony did not show the initial alkalinity preceding acidity. The cultures finally became alkaline, usually in 12–19 days. One culture, however, which remained acid for 9 weeks gave an alkaline reaction in 11 weeks.

Saccharose.—Acid reaction in 18 to 48 hours. With two exceptions the cultures remained acid for 2 months. Two colonies, however, became alkaline in 2 and 6 weeks.

Glycerine.—Acid reaction in 2 days. All except one colony, which showed a trace of alkali in 10 days, remained acid for 2 months. The culture of the exceptional colony was alkaline throughout in 30 days.

These sugar and glycerine agars afford a very excellent means of distinguishing *Bact. flaccumfaciens* from *Bact. phaseoli,* as cultures of the latter produce no acid but become alkaline in 2 to 3 days.

PRODUCTION OF AMMONIA, HYDROGEN SULFIDE AND INDOL

Ammonia was present in beef extract peptone broth (pH 7.0), tested with Nessler's solution 5 days after inoculation.

No hydrogen sulfide was formed in basic lead acetate beef extract agar cultures under observation for 3 weeks.

No indol present in Uschinsky + peptone cultures tested the 5th day with sodium nitrite and sulphuric acid. *B. coli* was used as a check.

REDUCTION OF NITRATES TO NITRITES

No reduction of nitrates in nitrate-peptone beef extract broth or synthetic nitrate solution in 2, 4, 5 or 11 days. Ammonia was produced in both the nitrate and the plain beef broth. The organism had not used the potassium nitrite in the peptone water + 2 p. p. m. potassium nitrite. Temperature 26° to 32° C. Sulphanilic acid and α-naphthylamine test used. Negative result also obtained with the potato starch water-potassium iodide-sulfuric acid test. No gas formed.

In earlier experiments the cultures were put into the thermostat at 32° C., which is about the optimum temperature for this organism. The long continued heat broke down the nitrate and in 7 days both the cultures and the uninoculated nitrate broth checks gave the nitrite reaction. This was confirmed by later experiments in which parallel sets of nitrate broth were inoculated, one being kept at room temperature, the other in the thermostat. The temperature of both places varied considerably during the experiment—the thermostat from 31° to 35° C., room temperature from 21° to 29.9° C., but when tested with sulphanilic acid and α-naphthylamine in 8 days all those in the thermostat, including the uninoculated checks, gave the deep pink nitrite reaction and all at room temperature remained colorless. *B. phytophthorus* was used as a check.

TOLERATION OF ACID

Tests were made in 17 beef infusion peptone bouillons ranging from pH 9.1 to pH 4.9. The optimum pH proved to be 8.0 to 6.2, clouding taking place in 9½ hours. The maximum tolerated acidity was about pH 4.9, some of the tubes clouding, others remaining clear. Growth occurred in pH 8.6 bouillons in two days, and some of the tubes clouded in the pH 9.1 bouillon in 5 days, at which time it had become pH 8.84.

TOLERATION OF NaCl

Growth occurred in beef bouillon containing 2, 3, 4 and 5 per cent NaCl. All but the 5 per cent had clouded in 42 hours. The 5 per cent sodium chloride produced quite an inhibitory effect, but in 5 days there was slight growth and transfers made from these cultures to beef broth produced clouding in 24 hours, showing that the vitality of the organism was unimpaired. Cultures were kept in the thermostat at 29°–31° C.

EFFECT OF FREEZING

Beef broth transfers from 24 hour old beef broth cultures were frozen in salt and ice for 15 minutes, 40 minutes and 1 hour, then thawed quickly and kept at room temperature (24° C.). All were clouded in less than 24

hours, though those exposed for an hour were less clouded than the checks.

LONGEVITY

Cultures have lived for 4 months at 1.5° to 2° C. Beef broth cultures at winter room temperatures (about 22° to 24° C.) were alive after 7 weeks, but cultures of the same colonies transferred at the same time from the same source to Thaxter's potato agar were dead at the end of this period.

The organism has been found alive and virulent on the seed, both under the seedcoat and on the exterior, at the end of five years, as already stated.

REACTIONS TO STAINS

Bact. flaccumfaciens stains readily with carbol fuchsin and Ehrlich's anilin gentian violet. It is Gram positive[4] (unlike *Bact. phaseoli*) and not acid fast. No capsules were demonstrated with Ribbert's capsule stain, although covers were stained from very viscid growth on 48 hour old beef agar slants, and from very viscid nitrate beef broth cultures.

SUNLIGHT TESTS

The only sunlight tests were made in January at Washington, D. C., on bright, cloudless days. Plates poured from beef broth cultures 24 hours old were exposed bottom side up. They were placed on ice and so slanted as to catch the direct rays of the sun. Exposures were from 30 minutes to 2 hours in length and in none of them were all the colonies killed. Half were killed in 30 minutes, two-thirds by 45 minutes and very few more by 2 hours. The 2 hour exposure, however, was later in the afternoon.

TEMPERATURE RELATIONS

Thermal death point.—The thermal death point is about 60° C. The majority of the cultures tested were killed by 57.5° and 59.9° C. but one colony survived both these temperatures. Beef bouillon tubes inoculated with 24 hour old beef broth cultures were exposed for 10 minutes in the water bath, readings being taken every half minute.

Maximum, minimum and optimum temperatures.—The minimum temperature is below 1.5° C. Tubes subjected to a temperature ranging from 0.9° C. to 1.5° C. remained clear for 2 weeks but became faintly clouded after 7 weeks. Others placed at a temperature ranging from 1.5° C. to 2° C. were clouded in 6 to 13 days. These cultures were alive at the end of 4 months.

The maximum temperature is between 36° C. and 40° C. In the thermostat, ranging from 38.5° C. to 40.5° C., there was no growth.

[4] *B. phytophthous* (Gram negative) used as check.

The optimum temperature is about 31° C. Growth occurred in less than 24 hours at 20°, 23.5°, 24°, 26°, 28°, 30.8° to 31.5°, 32° and 35.5° to 36° C., the most clouding at the end of this period being at 30.8° to 31.5° C. and the least at 35.5° to 36° C.

For these tests, peptonized beef broth was inoculated from peptonized beef broth cultures 24 to 48 hours old.

TABLE 1.—*Tests for differentiating Bact. flaccumfaciens from Bact. phaseoli*

Medium	*Bact. flaccumfaciens*	*Bact. phaseoli*
Congo Red agar	Very thin, scanty colony-wise growth	Copious, smooth, wet-shining growth
Cornstarch agar	Very weak diastasic action	Very strong diastasic action (Fig. 2)
Gelatin	Very slow liquefaction	Rapid liquefaction
Glycerine beef extract agar with brom cresol purple and phenol red	Acid reaction in 2 days	Alkaline reaction in 1–3 days
Litmus lactose agar	Pinkish cream growth: reduction and no bluing of litmus	Greenish yellow growth: bluing and no reduction of litmus
Milk	Slow formation of solid acid curd followed by separation of the whey and slow peptonization	Clearing 1–6 days; soft mobile curd, rapid peptonization preventing formation of a solid curd. Secretes a lab ferment (EFS)
Potato	Scanty to moderate growth accompanied by marked graying of the cylinder: very weak diastasic action	Very copious growth on fresh potato cylinders, finally so filling the water with slime that it remains in position when the tubes are inverted. Very strong diastasic action
String bean agar	Thin dull finely contoured growth	Good wet-shining growth
Sugar beef extract agars with brom cresol purple and phenol red. (dextrose, lactose, saccharose)	Acid reaction in 2–3 days	Alkaline reaction in 2–3 days
Thaxter's potato agar	Good but not copious, dry-shining, whitish, somewhat contoured growth	Copious wet-shining yellow growth
Whey agar	Good but not copious dry-shining somewhat contoured growth	Copious smooth, wet-shining growth
Gram's stain	Positive	Negative (EFS)

TECHNICAL DESCRIPTION

Bacterium flaccumfaciens Hedges.

Short, non-spore-forming rod with rounded ends, 0.6 to 3 μ x 0.3 to 0.5 μ, occurring singly or in pairs. No chains or filaments have been observed. Yellow (sometimes pale cream color), motile by means of a single polar flagellum 3 or 4 times the length of the rod, stained from 24 hour old beef agar slants with Casares-Gil's stain; aerobic; Gram positive, not acid fast, stains readily with carbol fuchsin and anilin gentian violet; produces neither nitrite nor gas in nitrate media; liquefies gelatin slowly; weak diastasic action; produces acid without gas from dextrose, lactose, saccharose, and glycerine; no capsules demonstrated; on beef agar slants produces moderate, flat, glistening, slightly contoured, translucent, cream to yellow, viscid, odorless growth; agar colonies round, smooth, wet-shining, flat, semi-opaque, finely granular with entire margins, appearing usually in 2 days; acid curdling and peptonization of milk and production of tyrosin crystals in old milk cultures; reduction of litmus in litmus milk; moderate to strong persistent clouding of nutrient broth, odor absent, sediment viscid on agitation; produces ammonia in beef broth + peptone; no production of indol in Uschinsky + peptone (5 days); no hydrogen sulfide produced in basic lead acetate agar; little, if any, clouding of the closed end of fermentation tubes containing peptonized beef extract broth containing dextrose, saccharose and lactose. Pathogenic to beans, causing dwarfing and wilt of seedlings and older plants and infection of the seed through which it transmits the disease.

For the sake of facilitating the diagnosis of *Bact. flaccumfaciens* a brief table of cultural tests is appended which will serve to differentiate it from *Bact. phaseoli* with which it has been confused in the past (Table 1.)

SUMMARY

This paper describes a seed-borne bacterial disease of beans causing wilting and death of seedlings, and wilting, dwarfing and sometimes breaking over of older plants. It is known to occur in South Dakota, Michigan, Montana, Virginia, Maryland, France and Germany.

The causal organism, *Bacterium flaccumfaciens* Hedges, has been isolated and infection repeatedly obtained through pure culture prick inoculations with subcultures from single colonies. Stomatal infections common in bean blight (*Bact. phaseoli*), do not occur.

Artificial infection also has been produced upon soy bean, but the disease is not known to occur naturally upon this crop.

Bean wilt is much less noticeable upon the pods than is bacterial blight (*Bact. phaseoli*) and might be easily overlooked, but the bright yellow

infected seeds of white seeded varieties are very conspicuous. They are common even in hand picked commercial seed following a season in which much wilt has occurred.

Observation and experiment have shown that the disease is transmitted through the seed in which the organism has been known to remain alive and virulent for five years.

As a control measure, the use of clean seed from wilt-free fields gives the most hope of success at present. The use of bean straw as fertilizer or feed should be avoided. Experiments on seed treatment and soil infection by *Bact. flaccumfaciens* are under way.

A technical dsecription of the causal organism and a detailed account of its cultural characteristics are given.

Some comparisons of *Bact. flaccumfaciens* and *Bact. phaseoli* are given, and a table of cultural tests for differentiating the two organisms is appended.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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EXPLANATION OF PLATES

PLATE I.

Pods and seeds infected with *Bact. flaccumfaciens*
Photo. by James F. Brewer

A. Badly infected seeds in pod of Navy pea bean which showed no sign of infection externally (compare B). Bacterial layer underneath the seed coat. Very infectious colonies plated from X. Coll. Michigan, Sept., 1922. About natural size.

B. Exterior of pod shown in A.

C. External infection on mature pods of Early Wonder. Olive greenish discoloration along suture. One of these might be easily overlooked. All contained infected seed. Coll. Michigan, Aug. 24, 1923. Natural size.

PLATE II.

Bean plants infected with *Bact. flaccumfaciens*
Photo. by James F. Brewer

A. Lazy Wife pole bean 2 months after prick inoculation below first pair of leaves. Whole top of main stem defoliated and dead. All leaves on lateral branch discolored and wilted. Pod contained diseased seed. ¼ nat. size.

B. Navy bean 9 days after prick inoculation below first pair of leaves. ¼ nat. size.

C. *Bact. flaccumfaciens* on 7 varieties of beans 4 weeks after inoculation by pricking. 1/6 ca. nat. size.

D. Checks for preceding.

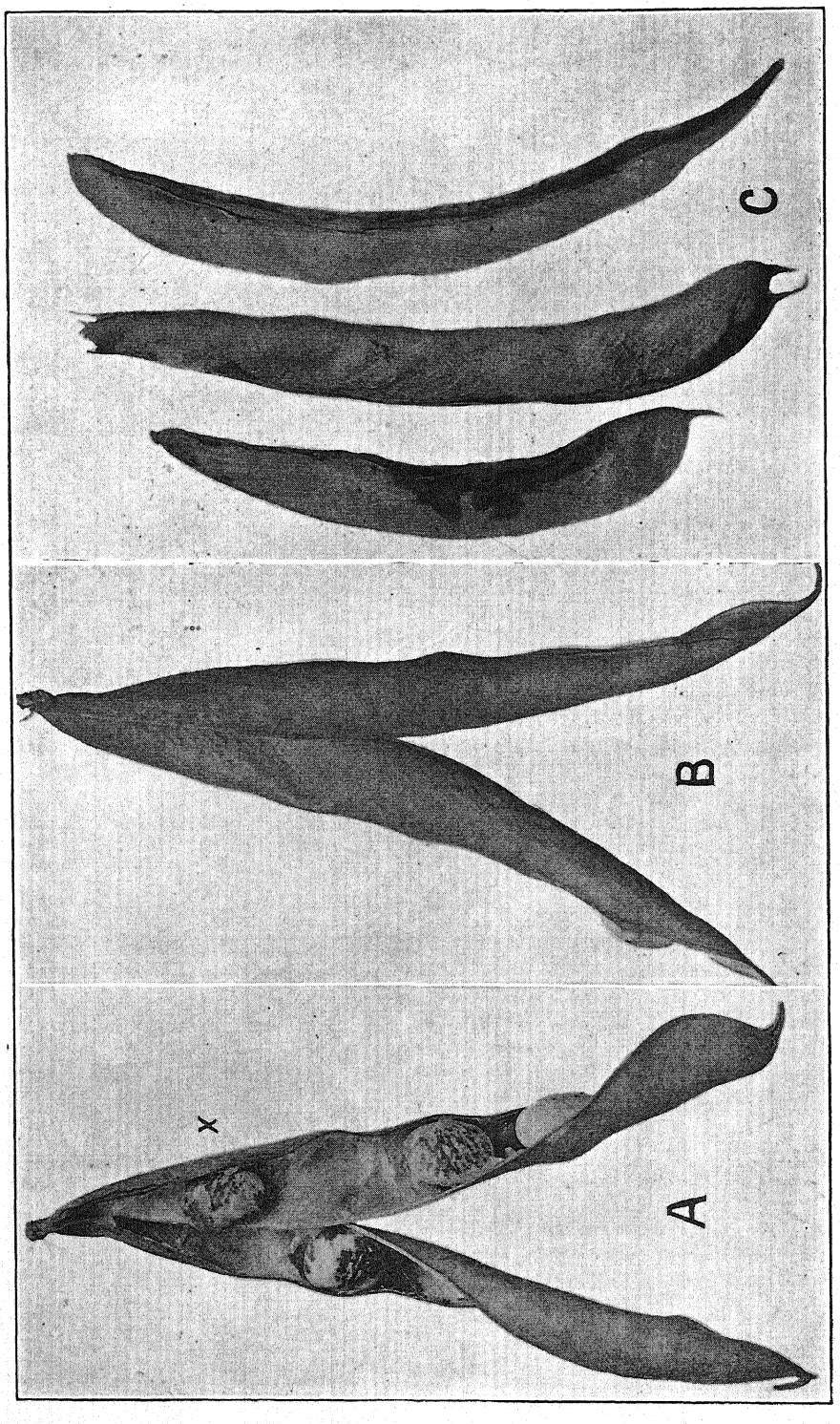
E. Wilt in Navy bean plants grown from diseased seed planted in sterilized soil in the greenhouse 5 weeks old. ¼ ca. nat. size.

PLATE III.

Pods and seed infected by inoculation with *Bact. flaccumfaciens*
Photo. by James F. Brewer

A. Pods of Lazy Wife pole bean 14 and 15 days after prick inoculation showing greater susceptibility of the seed. Natural size.

B. Pods of Lazy Wife pole bean 4 weeks after prick inoculations in the pedicels. Pods withering and yellowish green. Seed infected. Natural size.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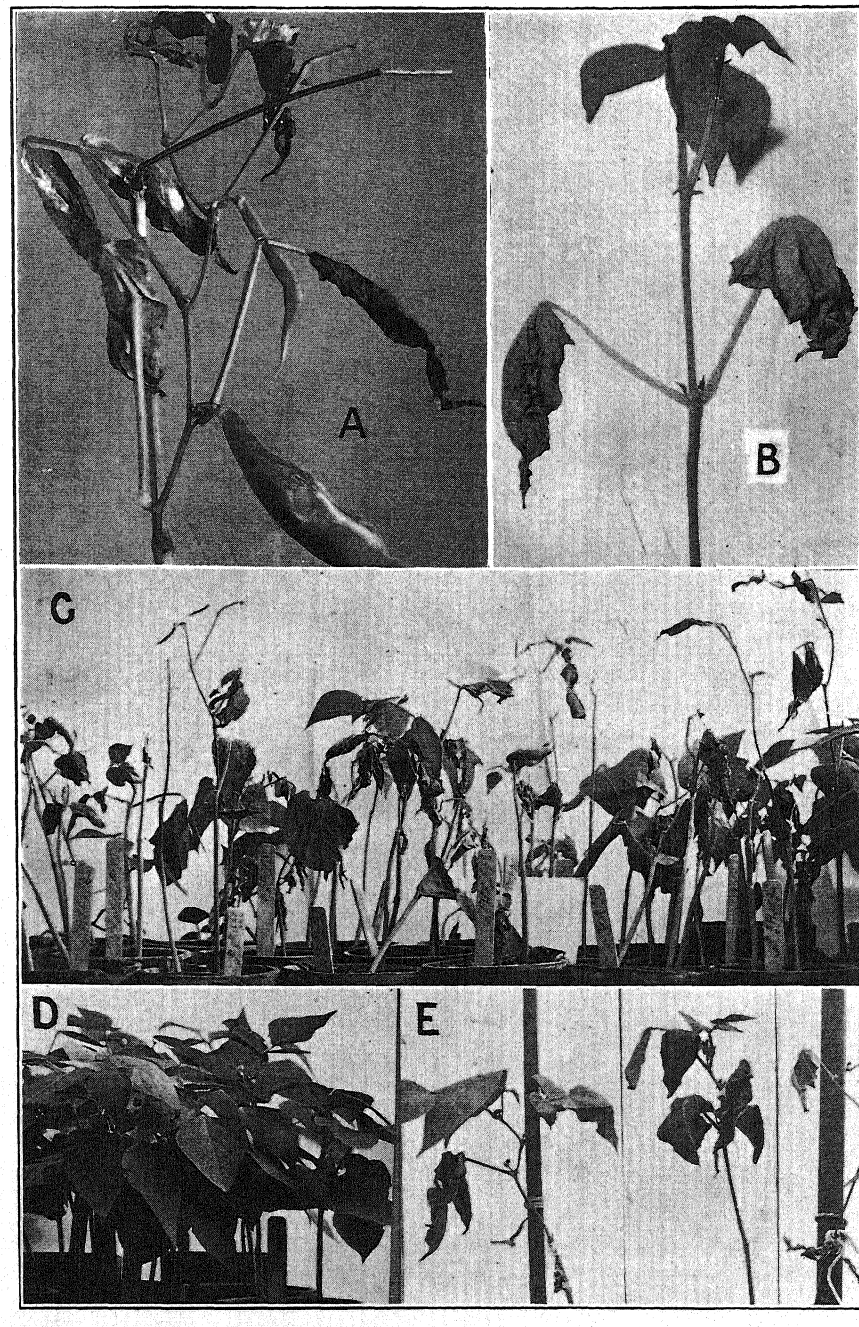
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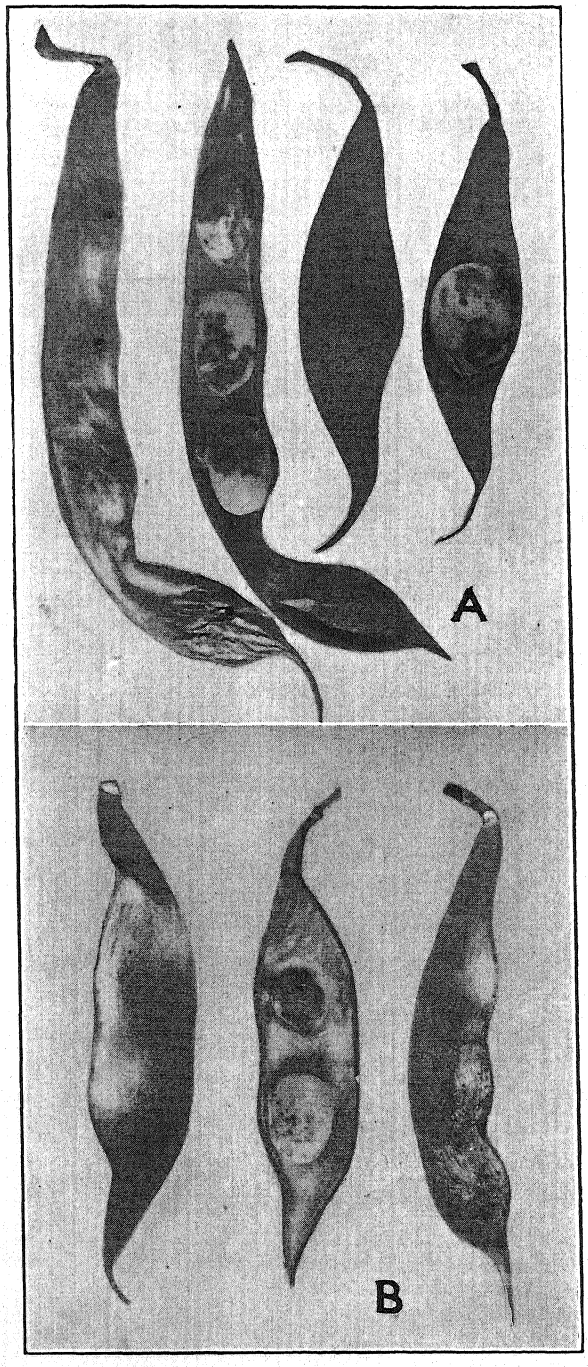
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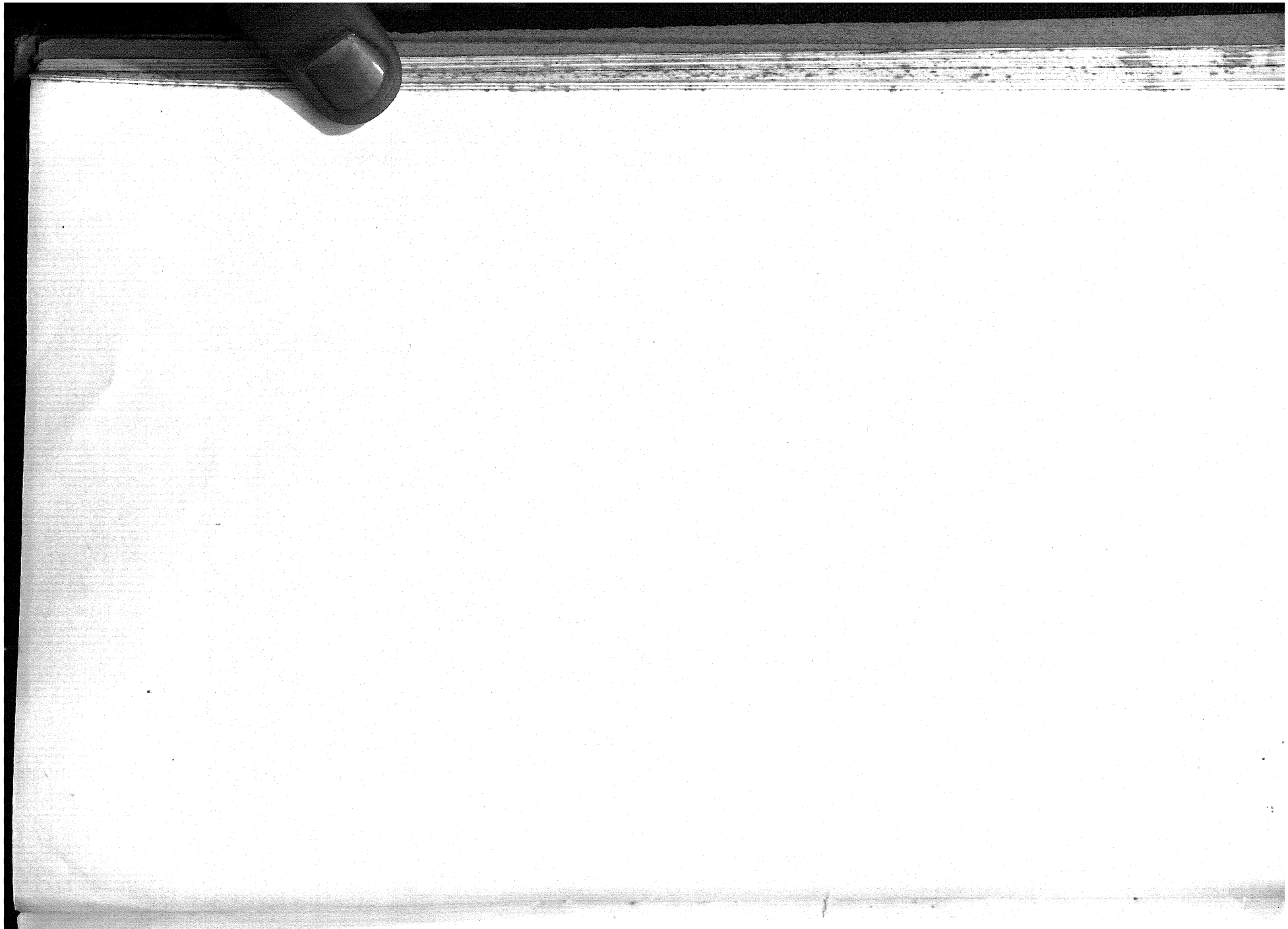

THE "BLACK DOT" DISEASE OF POTATO[1]

B. T. DICKSON

The increasing importance of the potato crop to human welfare needs no stressing, and one has constantly in mind the numerous diseases to which that crop is heir. This paper deals with a disease which is quite cosmopolitan in distribution and yet comparatively little known. Such a statement justifies the inference that it cannot be of prime importance at the moment; nevertheless, information regarding it should be available. The study has been carried on since the late summer of 1921, and further work is still necessary to determine accurately its economic significance. It is variously known as dartrose, anthracnose, foot rot, and black-dot disease.

HISTORICAL

In the summer and early autumn of 1921 at, and in the vicinity of, Macdonald College, the author observed a peculiar premature dying down of the tops of potato plants, and investigation revealed the fact that the condition of the plants was due to attacks by a fungus belonging in the *Colletotrichum-Vermicularia-Volutella* group. This was reported (13) under the name "black dot" disease, which is an excellent descriptive name given by McAlpine (20) to a similar affection of potatoes in Australia; and, since what appeared to be the same disease had been ascribed by Ducomet (16) to *Vermicularia varians,* the present author also ascribed it temporarily to that organism. However, certain growth characters of the organism rendered it necessary to make a more detailed study, the results of which appear herein.

A survey of the literature available gives the following references to the same or closely related diseases and organisms.

In 1825, Link (19) described *Exosporium maculans* as occurring on potato stems in France and Belgium. Fries, in his index to Volume III of the Systema Mycologicum (1829), gives *E. maculans* Lk. as a variety of *Vermicularia dematium* Fr. which also occurs on potato stems. While spore sizes are approximately the same, those of *V. dematium* are falcate, and those of *E. maculans* straight or but slightly curved. On this basis

[1] Read before the Botanical Section of the British Association for the Advancement of Learning at the Toronto Meeting, August, 1924.

Desmazieres (12) renamed it *Vermicularia maculans,* which was accepted by Fries in his Summa vegetabilium Scandinaviae . . . of 1849.

Berkeley & Broome (2), in 1850, reported *Vermicularia atramentaria* on decaying potato stems, forming inky-black patches and distinguished at once by its straight spores. Concerning *V. atramentaria,* the statement appears in the Sylloge Fungorum III, page 228: "*Verm. maculans* (Lk.) Fr. Summa V. S. p. 420, Kickx Fl. Fl. I, p. 405, valde affinis est, nisi eadem ac. *Verm. atramentaria* B. et Br."

Caspary (7) published an account of a similar organism found on potato, which he called *Steirochaete solani.* The genus *Steirochaete* A. Br. et Casp. is recognized in the Sylloge Fungorum, page 592, as: "verisimiliter totum genus cum *Colletotrichum* confluit" (see also Eng. u. Prantl. I, 403); and concerning *S. solani,* Caspary is quoted: "An eadem ac *Vermicularia maculans?*"

Between 1841 and 1859, Westendorp (28) noted *Vermicularia herbarum* on decaying stem of potato and other plants. In the Sylloge Fungorum III, page 226, the spore size is given as 3–4 μ by 20–22 μ, and the following statement occurs: "Longuis distat a *Verm. atramentaria* B. et Br. (? = *Verm. maculante* (Lk.) Desm.) ob perithecia majora ab laxe sparsa."

Hallier (17), in 1875, considered that a Krauselkrankheit of potatoes was due to an organism developing black sclerotial bodies clothed with black setae, which he named *Rhizoctonia tabifica.* This particular organism Pethybridge (23) showed to be the sclerotial stage of a *Colletotrichum,* which he therefore re-named *Colletotrichum tabificum* (Hall. pro parte).

In 1881 Saccardo and Roumeguère (25) recorded *V. orthospora* with spores 4 x 22 μ occurring on potato stems, and about the same time *V. eupyrena* with spores 4–4.5 by 20–26 μ occurring in "caule denique decorticate emortuo *Solani tuberosa*" was noted by Saccardo (24).

In 1886 Brunaud (6) described from the environs of Saintes, France, *Sclerotium solani* characterized by a black interior on dry potato stalks in winter. No spores were found, but it may be the same organism with which we are concerned in a slightly modified condition.

In 1894 Halsted (18), while working with potatoes, observed in connection with a stem blight a fungus which he considered to be *V. atramentaria* B. et Br., and regarding which he reported: "the amount it has to do with causing the destruction of the crop is an open question."

Recent attention was first drawn to the disease by Ducomet (16), who described the organism from France as *Vermicularia varians,* such species name being suggested, no doubt, by the final statement of his description: "Espèce variable, évoluant vers le *Phoma* ou inversement vers les *Colletotrichum* et *Gloeosporium.*" McAlpine (20) noted its occurrence in Aus-

tralia, pointing out that he could not find pycnidia in any of his specimens. In the same year, Manns (21) studied dry rot of the potato due to *Fusarium oxysporum* Schlecht, and observed: "it is common to find associated with the *Fusarium,* and likewise penetrating the tuber sometimes to a depth of one-fourth to one-half inch, a fungus of the genus *Vermicularia.*"

Doidge (15) reported from South Africa what is apparently the same disease as that described by Ducomet.

In 1915, O'Gara (22) published a full description of *Colletotrichum solanicolum* sp. nov. which he found in many potato fields in Salt Lake Valley, Utah. The organism confined its attacks mostly to underground parts, invading the cortex. The spore size given is 3.5–5 by 17–22 μ, and he notes that the organism is related to *Vermicularia.*

Taubenhaus (27), in the following year, dealt with silver scurf of the potato, and in the course of his studies decided that *Phellomyces sclerotiophorus* Frank is the same as *Vermicularia atramentaria* B. & Br., and the same as *Colletotrichum solanicolum* O'Gara. On page 355 he also noted that Dr. Manns pronounced his *Vermicularia* identical with *Colletotrichum atramentarium* (B. & Br.) Taub.

In 1916, Bewley (3) gave a description of a tomato disease which was attributed to *Sclerotium setosum* Bewley and Shearn. In correspondence Dr. Pethybridge informed the writer that he had submitted a culture of *C. tabificum* to Dr. Bewley, who had agreed that *S. setosum* was the same as *C. tabificum* (4).[2]

In 1923, Averna-Sacca (1) reported a destructive anthracnose of potato from Sao Paulo, Brazil. Affected plants could be pulled without effort, and under the cortex of subterranean parts *C. solanicolum* O'Gara occurred. The spore size given by Averna-Sacca differs somewhat from O'Gara's in width.

Brittlebank (5), in a discussion of tomato diseases, states that "black dot" root disease has caused considerable loss to tomato growers for the last fifteen years in Victoria, Australia. Recently Chaudhuri (9)[2] published an account of *Colletotrichum biologicum* also causing anthracnose of potato in Germany.

GEOGRAPHICAL DISTRIBUTION

From the above historical account it would appear that the same or closely similar diseases are cosmopolitan in occurrence. Localities from which reports are available to the writer include Quebec, New Brunswick, and Prince Edward Island in Canada (Dickson); New Jersey (Halsted,

[2] Received since the B. A. meeting.

18), Ohio (Manns, 21), Utah (O'Gara, 22), Florida in 1905, Oregon in 1916 (Dr. W. W. Gilbert in correspondence with the author), Pennsylvania in 1921–24 (W. A. McCubbin and Dr. Freeman Weiss in correspondence), Delaware in 1916 (Taubenhaus) and West Virginia in 1922 (Dr. N. J. Giddings, in correspondence) in the United States of America; England (Pethybridge); Ireland (Pethybridge, 23); Australia (McAlpine, 20); South Africa (Doidge, 15); France (Ducomet, 16); Brazil (Averna-Sacca, 1); Germany (Berkeley & Broome, 2, and Chaudhuri, 9); Italy (2 and 24); and Belgium (2 and 28).

SYMPTOMS

General. The first symptom is a slight yellowing of the foliage, beginning at the tips and gradually involving entire leaflets. At the same time a rolling may be noticed similar to that occurring in such stem infections as those caused by *Rhizoctonia solani, Fusaruim oxysporum,* and *B. atrosepticus.* This symptom has been well described by Crepin (10), who calls it "enroulement flasque" (flaccid rolling) and differentiates it from the condition in "leaf-roll" or "Leptonecrosis" of Quanjer. It may appear comparatively early in the season if infection is heavy and early, and if the weather becomes dry, but usually it is a midsummer to autumn symptom. In ordinary cases the foliage gradually yellows, droops, and dries up, so that affected plants present the appearance of dried mature tops some three weeks earlier than unaffected plants in the same plot. In dry periods, the shrivelling and browning of the foliage and stems occur much more rapidly, as was the case with certain plants in 1921. Crepin notes that where desiccation is relatively slow, axillary buds become swollen, and in certain varieties (Hollande du Gatinais, Violette du Forez, etc.) aerial tubers develop, sometimes abundantly.

Early infection, especially if accompanied by adverse environmental conditions, causes a definite dwarfing of parts of the plant developing under those conditions.

Underground Parts

Stems. The stem is commonly affected from just about ground level down to the parent tuber, the actual zone being determined by whether infection occurred from sclerotia on the parent tuber or from the soil or debris. The cortical tissues are invaded and broken down so that it is easy to scale them from the dry stem, as shown in Pl. IV, fig. 3. The mycelium fills the vascular tissues and appears on the inner circumference of the vascular cylinder. When the tissues are drying, it is not uncommon to observe, after peeling the remnant of the cortex, a delicate but definite

amethyst coloration, which is very marked on the inside of the vascular cylinder. Pethybridge (23) draws particular attention to this apparently usual coloration in material in England and Ireland.

When desiccation commences, the sclerotial bodies develop in varying numbers and with or without setae. They may not only line the vascular cylinder on both inner and outer periphery but often occur in considerable numbers on the inner surface of parts of the cortical cylinder, which by this time is loosened and easily removable.

Stolons. Similar conditions hold for stolons as described above for stems, but it is noticeable that relatively more lesions completely involving the tissues occur in stolons than stems. This is of importance in affecting yield, since stolons may be attacked at any stage of tuber development. Usually the lesion severing connection along the stolon is found from ½ to 1½ inches from the young tuber. When the tuber is dug thus incompletely matured, it can be seen to carry at its stem end a varying length of dried stolon which may or may not show sclerotia. The author has examined many potatoes from widely scattered sources, and in almost every case the adherence of a part of the stolon to a tuber is an indication of the presence of this disease.

Tubers. The tubers are affected in their development by the cutting off of food as a result of stolon lesions, and, since this may occur at any stage in growth, it is possible to find tubers just commencing to swell, through all grades to large mature potatoes separated from the parent system. If very small, the tubers soon rot or shrivel up, but larger potatoes may remain until harvest and show no other symptoms than small size and the attached fragments of stolons. On the other hand, such tubers may be covered on the upper surface, all over, or in patches, with sclerotia. This may also happen with a tuber of which the stolon is unaffected. The aggregation of sclerotia over the surface varies from rather scanty to close; for example, on one tuber the estimate was approximately 300 in a square inch.

On freshly dug white potatoes, the writer has seldom observed the greyish areas, somewhat resembling silver scurf, mentioned by Ducomet (16) and Crepin (10), and because of which the former named the disease "Dartrose." Ducomet states that this condition is more easily seen after the potatoes have been stored some time, and this the writer can corroborate. Pigmented varieties have not yet been studied, but both the above-mentioned French authors agree that on purple and red varieties the rather rough decolorized plaques are easily seen.

Roots. The roots are attacked in all stages of development, and they also are decorticated. When the roots are pulled, it is usual to observe the

broken naked vascular cylinder with attached fragments of desiccated cortical tissues carrying sclerotia. The root lesions account for the ease with which one may pull up the stems of affected plants, since the roots break about two inches from the stem.

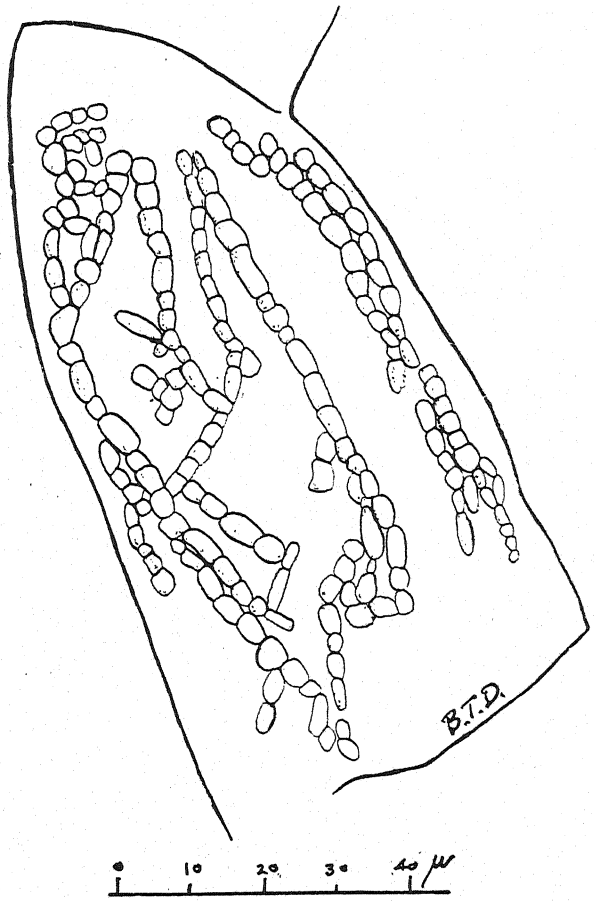

FIG. 1. Mycelium in trichome cell of completely parasitized potato plant. Camera lucida drawing.

PATHOLOGICAL HISTOLOGY

The author's studies of the pathological histology have so far not been systematic, but in general they confirm the careful work of Ducomet (16). In one notable case, a greenhouse plant artificially inoculated indicated a complete parasitism. The mycelium apparently grew rapidly up the vascular cylinder of the stems into the leaves, and invaded even the trichomes (Fig. 1).

ETIOLOGY

Ducomet (16) affirmed: "Le parasitisme de notre éspèce n'est pas douteux." He pointed out (p. 42) that when Delacroix was studying his *Bacillus solanincola* in 1901 he also found a *Fusarium* which appeared to correspond with *F. lycopersici* Sacc., and a second fungus which he thought ought to be called a species of *Vermicularia*. Manns (21) found a *Vermicularia* present to the extent of 10.3 per cent among his isolations when working with *Fusarium oxysporum* Schlecht. He states (p. 316): "When used alone it brings about no disease symptoms. If it assists any in bringing about the disease, its work is that of a semi-parasite which follows the openings made by the *Fusarium*. It is almost invariably present on the underground stems and roots of sick plants in the field, and upon tubers which are carrying internally the *Fusarium* fungus, showing plainly by means of the hand lens at the point of the stem connection. The writer has many times taken it from internal parts of the roots and underground stems of potato plants which were showing only the first symptoms of *Fusarium* blight."

Crepin (10) finds that it is common on plants killed by attacks of late blight in late August. In Bretagne and at Grignon: "il est vraisemblablement saprophyte et il nous apparait comme étant très répandu."

In the original isolations of 1921, the author also found in many plates that a *Fusarium* (probably *F. oxysporum*) appeared in the cultures, and, because of its rapid growth, that organism overran the black-dot organism. The latter invariably appeared, however, and the fact remains that in approximately half the plates out of some eighty it came up pure or contaminated with bacteria, and in three cases contaminated also with *Rhizoctonia*.

Dr. G. H. Pethybridge was good enough to send, early in 1924, some material collected in Lancashire, England, in December, 1923. Part of this was used for isolation, and a *Fusarium* appeared in ten of the cultures out of twelve, the two others giving pure plates of the black dot organism.

Undoubtedly, therefore, a *Fusarium* is very frequently associated with our present fungus, but so far the writer's work has led him to believe that the *Fusarium* is secondary or associated with the *Colletotrichum*.

Experiment 1

During the fall of 1921 careful collections were made, from affected Green Mountain plants, of tubers carrying numerous sclerotia. They were washed in mercuric bichloride solution (1 part in 1,000), for 30 minutes, and eleven hills were planted in the spring of 1922 in the experimental

plots of the Department of Botany at Macdonald College. The plants grew poorly; they were stunted and weak, and showed all symptoms of dartrose. By September 18, all the tops were dead, while adjoining rows were full of green tops. The largest tuber obtained was 2 inches long, and the majority were about one inch. Every stem exhibited abundant sclerotia, and many showed the amethystine coloration described by Pethybridge (23). There were ample evidences of stolon infection and the subsequent development of new stolons which in turn were attacked. The tubers were plentifully besprinkled with sclerotia. Isolations from the stem gave cultures of the organism often contaminated by bacteria but rarely by *Fusarium*.

Experiment 2

At the same time a plot of 64 Green Mountain potatoes, with eight hills per row, was planted. On July 4th, when the plants were about 3 inches high, the following inoculations were made:

Row 2, with the organism isolated by the author.

Row 4, with cultures of *C. atramentarium* (by kindness of Dr. Detmers).

Row 6, with cultures of a *Colletotrichum* from West Virginia (sent by Dr. Giddings).

The inoculations were made by carefully removing the soil to a depth of about three inches and placing on the exposed stem an agar culture about one-quarter inch in diameter. Every effort was made to avoid injury to the stem and roots. The results observed on September 20 were as follows:

Row 2, seven plants dead, all stems with sclerotia.

Row 4, six plants dead, all stems with sclerotia.

Row 6, four plants dead, all stems with sclerotia.

Rows 1, 3, 5, 7 and 8, plants all green; free from disease.

Isolations from inoculated plants again gave cultures of the black-dot organism contaminated by bacteria, but only in three plates out of eighteen by *Fusarium*. The similarity of the organisms used will be discussed below.

Experiment 3

In the field it is always possible that *Fusarium* spp. are present in the soil; in order to obviate this, a set of greenhouse tests were started on February 21, 1924. Green Mountain potatoes were treated by the hot formaldehyde method and planted in pots. Both pots and the soil therein had been thoroughly autoclaved. On March 15, when the plants were about 3 inches high, they were inoculated. An examination of the plants on June 1 gave the results shown in table 1.

TABLE 1.—*Results of inoculating potato plants growing in pots of sterilized soil*

No. of plants inoculated	Inoculum	Source	Results
2 (A)[a]	Quebec organism	Dickson	Plants yellowed, wilting unless well watered; stem, stolons, roots, and tubers with sclerotia
2 (B)	do	do	do
2 (A)	Saltation	Dickson (14)	Plants showed symptoms but very few sclerotia
2 (B)	do	do	One plant do
2 (A)	*C. tabificum*	Isolated from material sent by Dr. Pethybridge	Two plants typical as above
2 (B)	do	do	Two do
2 (A)	*V. varians*	Isolation by Cavadas	Two do
2 (B)	do	do	One do
2 (A)	*C. atramentarium*	Isolation by Dr. Detmers	One do
2 (B)	do	do	Two do
2 (A)	*Collecotrichum* sp.	Isolation by Dr. Giddings	Two do
2 (B)	do	do	One, slight infection; no obvious symptoms

[a] Series (A): Inoculations with injury by scalpel to stem.
" (B): Without apparent injury.

In the above experiment every effort was made to avoid contamination by other fungi, and isolations from the soil at the end of the test gave no indication of the presence of *Fusarium* spp. The similarity in results obtained with the different cultures will be dealt with later.

Field tests were made during the season of 1924 in a plot which had not been planted with potatoes for at least twelve years. The ground was a heavy clay loam, which was very wet during the spring and very dry during June. Disease-free seed was planted on June 9; and on July 3 and 4, six hundred and thirty hills were inoculated as indicated in figure 2. Each plant was exposed just below ground level with as much avoidance of injury as possible, and a small mass of potato dextrose agar culture or

dry stem material was applied to the stems, which were at once recovered.

Examination was made on Sept. 20, 1924, when it was comparatively easy to detect prematurely dead tops. The crosses in the diagram (Fig. 2) indicate the affected plants. It was noteworthy that in every series an amethystine coloration was present on most of the diseased stems. In the case of the saltation there were few sclerotia, but they were definitely pres-

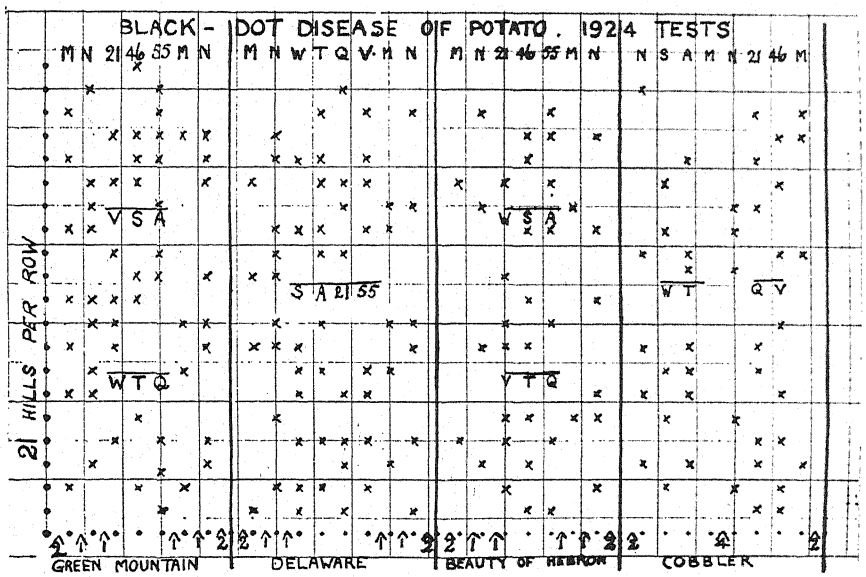

FIG. 2. Diagram of the 1924 experimental plots in which four varieties of potato were inoculated with eleven different black-dot cultures. Crosses (x) indicate infected plants. 1, 2, 4 = number of check rows between inoculated rows; M = fragments of stems with sclerotia collected during 1922 and kept dry in a cool place; N = fragments of stem with sclerotia collected during 1923 and kept dry in a cool place; S = saltation; V = subculture by author from original by Cavadas through Dr. Foex and Dr. Pethybridge; Q = organism isolated in Quebec; T = isolation by author from Lancashire, Eng., material sent by Dr. Pethybridge; W = subculture from original by Dr. Giddings from West Virginia; A = subculture from original by Dr. Detmers from Ohio; 21 = subculture from original by Dr. Weiss (from wilted plant); 46 = subculture from original by Dr. Weiss (stem-end rot of tuber); 55 = subculture from original by Dr. Weiss (from wilted plant).

ent. It would appear from this one season's inoculations that Green Mountain and Delaware are somewhat more susceptible than Beauty of Hebron and Cobbler. Except with the saltation, it was impossible to distinguish any one series from another, either by the effects on the stem, by coloration produced, by sclerotia, or by spores. From this it is justifiable to infer that the various inocula used from such widely distributed sources are of the same cosmopolitan organism. This confirms cultural studies on these isolations carried on by Scott (26).

Pethybridge (23) inoculated healthy potato stalks and tubers through wounds, but, beyond a strictly limited growth in the wound, no development occurred; and no trace of any kind of rot was set up. He concluded: "there may possibly be special conditions under which it behaves as a parasite or at least as a feeble parasite."

Cavadas (8) carried out some tests in the greenhouses of the Station de Pathologie Végétale of Paris. In two pots the soil was inoculated with conidia, in two others with sclerotia; two tubers with conidia, two tubers with sclerotia; two plants on the wounded stem at ground level when 8–12 cm. high; two with conidia and sclerotia without wounding when the plants were 8–12 cm. high; two plants were left as checks.

Characteristic symptoms were evident in the plants from inoculated soil and tubers. With the wounded plants the symptoms were less definite, and it appeared that the plants reacted against the parasite to limit its action on the inoculated part. The plants inoculated (disseminé) without wounding remained healthy. Cavadas concludes that *Vermicularia varians* Duc. becomes a true parasite and can give rise to serious losses only when it attacks the plant in early stages of growth. In other cases it is inoffensive, but it can help in the destruction of the plant if some factor diminishes resistance.

With these conclusions the author can generally agree, as a result of the experimental work so far completed. Nevertheless, without further and wider observation it is not possible to determine the economic importance of the organism, although the author believes it to be more important than is generally believed.

MORPHOLOGY OF THE ORGANISM

In the course of three years' culturing, the organism has been grown on various media, among which the best were potato-dextrose, Czapek's, oatmeal, and cornmeal agars. Since potato-dextrose was used most, it will be the one considered in the following description.

From single spores the first indication is a white, silky, superficial mycelium, spreading at the rate of about 8 mm. a day at 21° C. The hyaline cells are 10–35 μ long by 3.5–5 μ in diameter. In about four days an amethystine tint, most pronounced in the centre of the culture, begins to appear in the submerged mycelium. In a few plates a yellow coloration may be evident. Dr. Detmers, in correspondence of March 30, 1923, concerning *C. atramentarium,* notes that in media of high sugar content "the conidial masses show a characteristic yellow, almost apricot yellow color." Our media contained one per cent of sugar, and the yellow or amethyst

coloration was always a sign of superabundant spore formation. In about six days sclerotia begin to appear in concentric rings, and the superficial mycelium becomes less apparent until the whole plate is covered with sclerotia. On Czapek's and cornmeal agars the sclerotia are more widely placed than on dextrose agar, on which medium they become so numerous that they tend to coalesce, and often eventually form an almost solid black cake. Conidia germinating and growing on the surface of this again give a coating of white mycelium which is usually rather patchy in development.

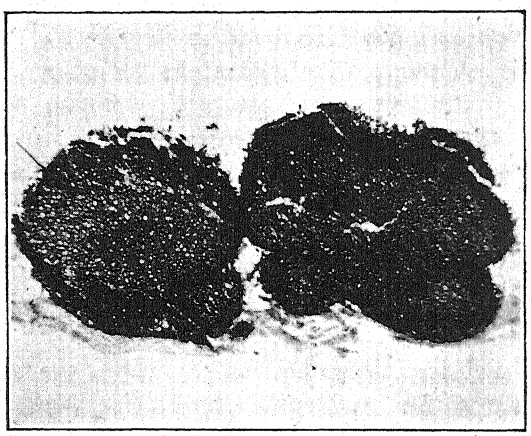

FIG. 3. Photomicrograph of cross-sections of sclerotia of Quebec organism. No evidence of pycnidia. Cultured on potato-dextrose agar. ×75.

The black sclerotia, with or without setae, vary in size from 100 microns to ½ mm. (Fig. 4). When young they contain oil, and when mature are made up of a dark, thin-walled pseudoparenchymatous tissue (Fig. 3), the cells of which are 4–9 μ by 10–14 μ in size. No pycnidia have ever been observed in our cultures.

The setae develop as usual with *Colletotrichum,* and vary in length from 80 to 350 μ, tapering from a somewhat swollen basal cell of 5–7 μ diameter, hyaline at the tip, but darkening with age and showing two to four septa. As many as 70 setae have been counted on a single sclerotium. They do not always appear in culture, especially if the organism has been subcultured for a considerable period. In nature they may not always be present, but on the other hand they can often be seen with the naked eye, especially on the outer periphery of the vascular cylinder.

The conidiophores develop free or in palisade layers and are subhyaline, 10–30 μ long, cylindrical, tapering to slightly clavate, sometimes curved, occasionally branched and from 0 to 3-septate.

Conidia are often developed in tremendous numbers, especially in the case of the saltation (14). The writer has counted as many as 174 spores, lying like logs in a timber raft, which apparently had developed from 15 conidiophores. They are pink in mass (except saltation, in which they are yellow), hyaline individually, continuous, sometimes slightly curved, somewhat attenuated at the basal end and round at the apical end, 3–7.5 μ by 17.5–22 μ, and 1 to 3-guttulate.

The germ tube usually develops at the apical end or just to one side, and appressoria are common in hanging-drop cultures. At times, what appears to be a septum is seen in the conidium just at germination, and a noticeable feature is a swelling of many of the spores to almost twice their original diameter, probably due to hydrolysis of some constituent of the spore contents with the consequent absorption of water.

Since this paper was read, the author has received from Dr. Chaudhuri a culture of his recently described *C. biologicum* (9). Dr. Chaudhuri differentiates his organism from that of Dr. Pethybridge by the absence of amethystine fluorescence. Remarkably enough, our first subculture made on oatmeal agar developed a beautiful amethyst coloration, and this has since persisted in subcultures. Chaudhuri also points out that the sclerotia are larger than those of *C. solanicolum* and the conidiophores longer. The present author has found that sclerotial size is somewhat unreliable, inasmuch as considerable variation may occur; and conidiophores as long as 30 μ have been found in subcultures from Dr. Detmer's isolation. He also finds two sizes of conidia, *viz.*, 5 x 22 μ and 5 x 14 μ. So far, the average size of conidia obtained in our subcultures is 3.75 x 17.5–20.6 μ, and we have not observed any definite differentiation into the two groups, although some spores are but 10–15 μ long. Dr. Chaudhuri remarks on the resemblances between *C. solanicolum, C. atramentarium, C. tabificum,* and *C. biologicum,* and as a result of preliminary studies on *C. biologicum* it appears to the present author to be so closely similar as to justify its being regarded as the same.

TAXONOMY

Link (19), in 1825, described *Exosporium maculans* as occurring in winter on dried stems of herbaceous plants; and Fries, in the index (p. 89) to Volume III of the Systema Mycologicum, noted it as a variety of *Vermicularia dematium* Fr. In 1828, Desmazières (12) named it *Vermicularia maculans,* for which the following description appears in Sylloge Fungorum **3**: 228:

"*Vermicularia maculans* (Link) Desm. Exs. n. 339, *Exosporium maculans* Link Sp. plant. Fung. II p. 123.—Peritheciis innatis, minutis, aggregatis, dein confluentibus et matricem nigrificantibus, rotundatis, applanatis, setulis concoloribus, sparsis, fasciculatis, apicem versus attenuatis, divaricatis obsitis, denique apice hiantibus; sporulis (paucis) elongatis vel oblongis, rectis, subobtusis.
Hab. in caulibus plantarum herbacearum, praecique *Solani tuberosi* et Utricae urentis in Gallia et Belgio."

Berkeley & Broome's (2) *Vermicularia atramentaria* appeared in 1850, and the technical account in Saccardo's Sylloge Fungorum, p. 227, is given herewith:

"*Vermicularia atramentaria* B. et Br. Ann. N. H. n. 430.—Effusa, gregaria, atra, maculiformis; peritheciis minutis superne setulis longis rectis, basi vero fibris repentibus, subepidermicis cinctis; sporulis minutis, cylindraceis, brevibus, utrinque nucleatis, (plasmate utrinque retracto, Berk.).
Hab. in caulibus *Solani tuberosi* in Britannia, Italia, Gallia, Belgio, Germania.—*Verm. maculans* (Link) Er. Summa V. S., p. 420, Kickx Fl. I. p. 405, Valde affinis est, nisi eadem ac *Verm. atramentaria* B. et Br."

The statement regarding the close similarity, if not identity, of those two organisms is noteworthy. By the kindness of Dr. A. W. Hill, Director of Royal Botanic Gardens at Kew, England, the author has been able to compare them, and finds that the only difference discernible in the exsiccati specimens is that of spore shape; but this appears to be sufficiently definite to separate them. Those of *V. atramentaria* (*C. atramentarium*) are straight cylindrical, rounded at the apex, and slightly tapering at the basal end, while those of *V. maculans* (*C. maculans*) are straight to slightly curved, appearing from a lateral view short glume-shaped in outline.

Taubenhaus (27) studied the specimens of *Vermicularia atramentaria* of the New York Botanical Garden, and, concluding that the fungus fits in better with *Colletotrichum,* named it *C. atramentarium.* His own cultures were submitted to Dr. O'Gara, who pronounced it the same as the latter's *C. solanicolum.* Taubenhaus was studying silver scurf (*Spondylocladium atrovirens*) of the white potato, and held the view that the sclerotial bodies he found were the same as *Phellomyces sclerotiophorus* Frank, which he therefore decided to be an invalid type, since his sclerotial bodies gave rise to the *Colletotrichum* cultures.

In material obtained from New Brunswick the present author was fortunate enough to culture from the same tubers both the *Colletotrichum* and *Spondylocladium*—the latter from sclerotial bodies which were undoubtedly stromata of *Spondylocladium atrovirens* Harz. It is thus apparent that both sclerotial bodies may be present on the same tubers, and

we therefore cannot agree with Taubenhaus in his conclusion regarding the invalidity of *Phellomyces sclerotiophorus* Frank, although this does not contradict his view that the *Colletotrichum* is the same as *V. atramentaria* B. & Br.

Crepin (11) notes that "Dartrose et gale argentée se presentent sous un aspect assez analogue et il faut quelque attention pour les distinguer."

Since the B.A. meeting the author has had the opportunity of comparing exsiccati specimens from Kew of *V. herbarum* and *V. orthospora* (= *C. orthosporum*). He was unable to find setose sclerotia, conidiophores and

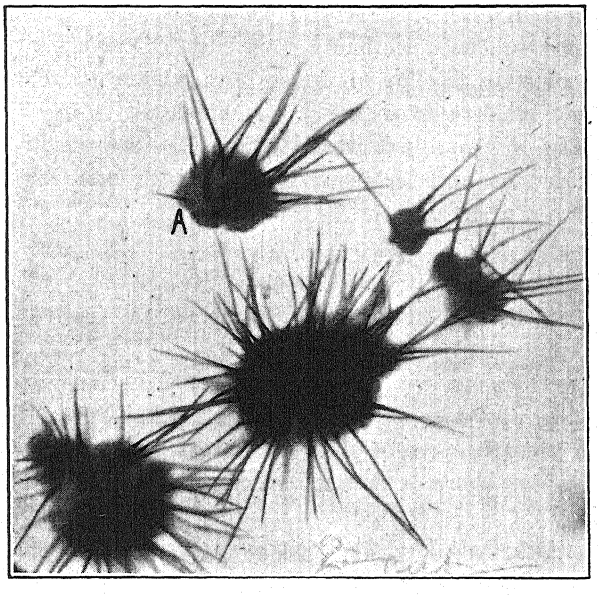

FIG. 4. Sclerotia with setae, Quebec organism. Tips of spores visible at A. ×100.

conidia of *V. herbarum,* and therefore unable to decide regarding that organism. *V. orthospora* was closely similar in all respects to our organism, except that all the sports were typically larger. It is obviously closely related, but, judged by spore size, is not identical.

Summing up such taxonomic studies as have been made, it may be stated:

1. That the organism causing black-dot disease, anthracnose, or foot rot of potato is properly known as *Colletotrichum atramentarium* (B. & Br.) Taub.

2. That the following organisms are the same and therefore their names synonymous: *C. tabificum* (Hallier pro parte) Pethy., *Rhizoctonia tabifica*

Hall. (17), *C. solanicolum* O'Gara, *Vermicularia varians* Duc., *Sclerotium setosum* Bewley and Shearn, and probably *C. biologicum* Chaud.

3. That *C. orthosporum* (Sacc. et Roum.) nov. comb. is very closely related.

CONTROL

No definite experiments relating to control have been undertaken, but from general observation certain facts are plain. The disease often occurs in poorly drained sections of otherwise healthy fields of potatoes. The avoidance of such areas is therefore advisable. The sclerotia of the organism may remain in plant debris, and perhaps in soil, for more than one year; and, if the soil becomes badly contaminated, rotation must be practised. Since sclerotia develop on tubers and adherent stolon pieces, seed-tuber treatment, preferably with hot formaldehyde, is necessary. Insufficient work has been done to warrant conclusions as to varietal susceptibility, but Green Mountain and Delaware appear to be more susceptible than Beauty of Hebron and Cobbler.

SUMMARY

1. Black-dot disease, dartrose, anthracnose, or foot-rot of potato is widely distributed.

2. The symptoms are similar to those of other diseases due to organisms attacking cortical and vascular tissues at and below ground level, but not so likely to be acute because of the rather weak parasitism of *C. atramentarium*. On tubers there is frequently considerable simulation of silver scurf.

3. *C. atramentarium* is a weak parasite, attacking mainly debilitated plants or young plants which succumb in early maturity.

4. Comparative studies show that organisms from England, France, Germany, United States of America and Canada are the same.

MACDONALD COLLEGE,
McGILL UNIVERSITY,
CANADA.

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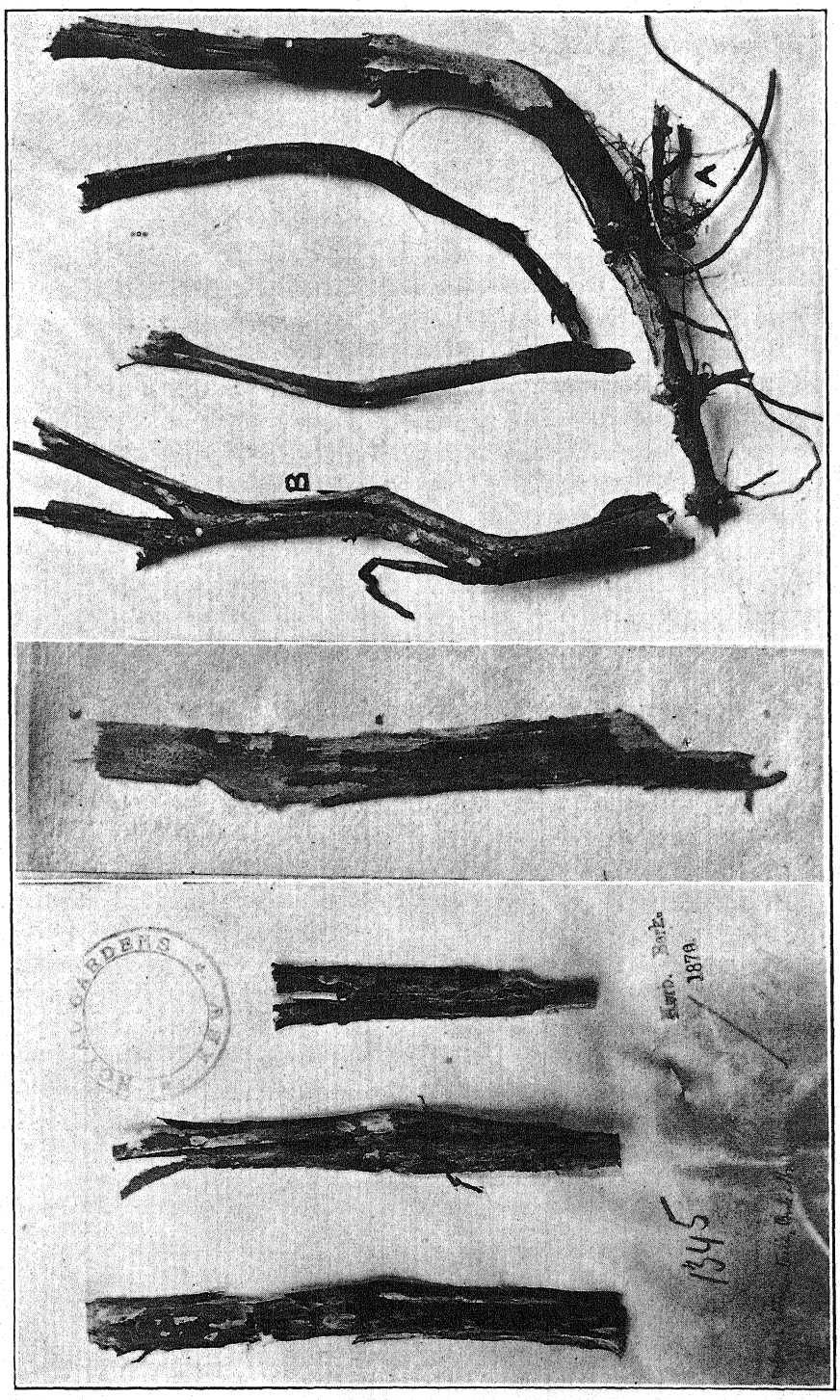
PLATE IV

1. *V. atramentaria* Berk. and Br. Note individual sclerotia in places and confluent sclerotial masses in others. Mount discolored at bottom.

2. *V. maculans* (Lk.) Desm. Closely similar to *V. atramentaria*.

3. 1923 collection of Quebec material. Decorticated tissues at A. Sclerotia confluent on stem at B.


A
B
1
2
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1845
1879

PLATE IV

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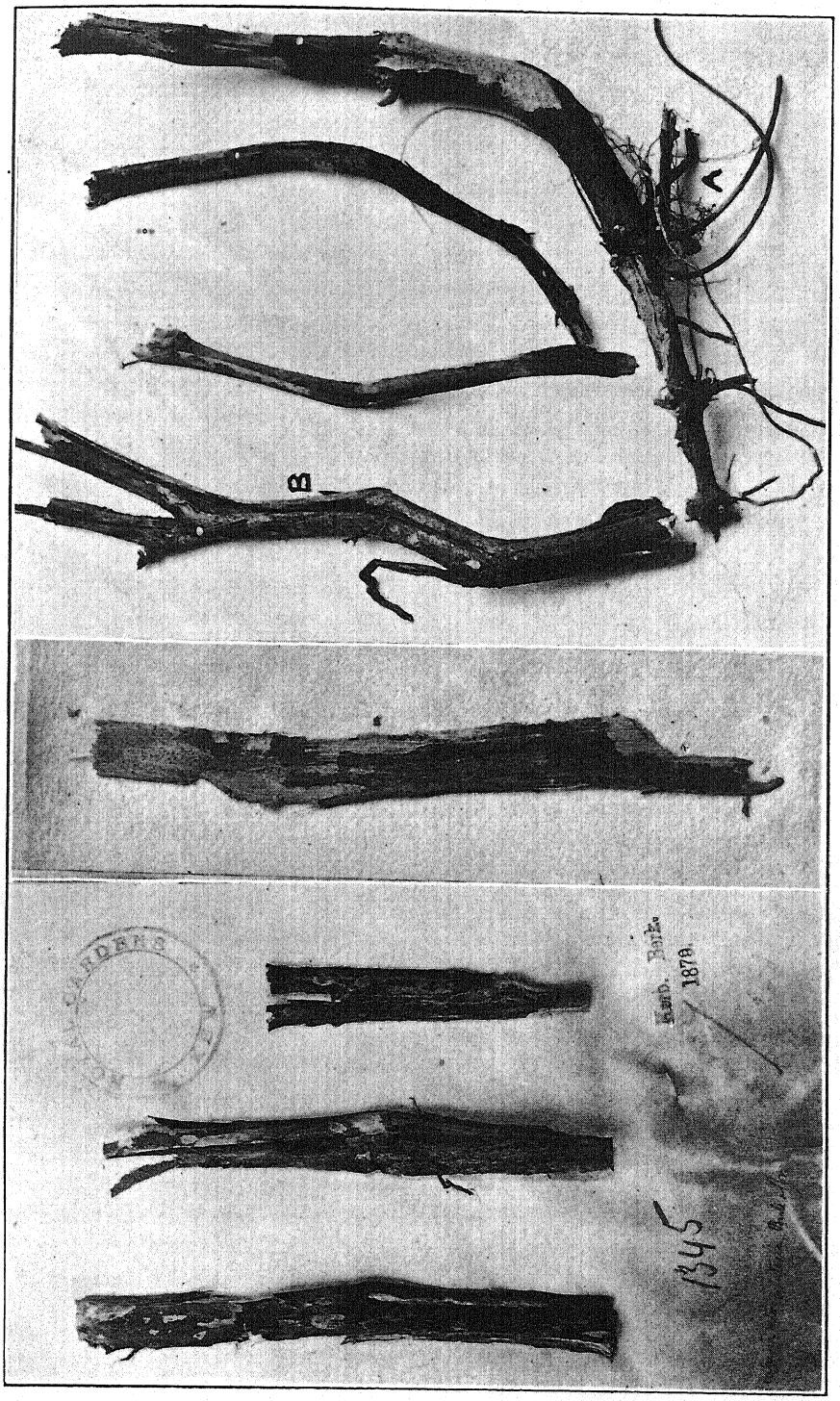

1 2 3

THE VARIABILITY IN THE BLACK ROT FUNGUS OF THE APPLE

J. S. Cooley and E. Aline Fenner

The question of the constancy of such physiological processes as the pathogenicity of a fungus is always an interesting and important one. It is particularly so with those fungi occurring in widely separated regions and having a number of unlike host plants. The black rot fungus of the apple, *Physalospora malorum* (Berk.) Shear, has a wide geographical range, for it occurs about as far north in Canada as the apple is grown and as far south in the United States as southern Georgia. It attacks the fruit and leaves of the apple and other pomaceous plants and lives as a saprophyte on the dead twigs of a great many other plants (4, 5). Since this fungus lives and thrives in unlike environments and on many different hosts, it is especially fitted for a study of variation in pathogenicity. This paper reports data on the behavior, when inoculated into the apple, of different cultures of the black rot fungus from the same locality and also from different and widely separated regions. It also reports data on the ability of the black rot fungus to produce rot after having been grown in culture for one year.

While engaged in the work reported by Brooks and Cooley in an earlier paper (1), there was some evidence that *Physalospora malorum* from different sources exhibited variability in its capacity to rot apples. It was not possible to get any further evidence on this point at that time. In the summer of 1923 experiments were begun to determine: (a) whether different cultures of *Physalospora* from the same locality differ from one another, and (b) whether *Physalospora* from one locality differs from that from another. In this way, fungi that may have been living for a number of generations in a certain environment may be compared with those from a distant locality where a very different environment exists.

The general impression seems to prevail among some workers with this organism that there are several strains possessing different degrees of virulence. Miss Walker (7) reports two strains of *Physalospora* that are very different pathogenically and morphologically. Brooks and DeMeritt (2) report three strains that show variability in pathogenicity and are somewhat unlike morphologically. Hesler (4) also mentions several strains that vary in their ability to produce rot.

The work of Stevens (6) shows that *Physalospora* produces a stroma on currant canes somewhat different from that produced on apple twigs. The spores, however, were remarkably constant on such unlike substrata.

In order to determine the variability of different cultures, not only from widely separated sections but also within a particular region, cultures were obtained from a number of different localities. Ten or more apples that appeared to be affected with black rot were secured from a given locality and the fungi isolated. Groups of about 10 cultures were thus obtained from Cornelia and Fort Valley, Ga.; Bentonville, Ark.; U. S. Agricultural Experiment Farm at Arlington, Va.; Middletown, Roanoke, Winchester and Covesville, Va.; Rockville and Snow Hill, Md.; Salem, Ind.; Mont Alto, Pa.; Durham, N. H.; and Guelph, Ontario. In some cases we were unable to obtain as many as 10 cultures from a locality. Of the 155 cultures that were used in the inoculations, 97 were isolated in 1923 and 58 in 1924. All the cultures were kept under the same environment after isolation. Isolations were made by slicing off a portion of the apple through the sound and diseased tissue and removing a bit of the decayed pulp from the margin of the rot.

The fruit for inoculation was washed thoroughly, disinfected in a bath of 50 per cent ethyl alcohol, and then rinsed in sterile water. Uniformity in the fruit of the different lots was secured by selecting as many apples of a certain size and degree of ripeness as there were cultures, placing one in each moist chamber, and repeating this until eight to ten apples were placed in each receptacle. The apples were inoculated by inserting a bit of the mycelium in the apple by means of a spear-pointed, sterile needle. After the fruit was inoculated, a piece of wet towel paper was placed in each moist chamber to maintain a humid atmosphere. The moist chambers were all stacked on the same table in the laboratory, where the temperature ran from 20° to 26° C. Previous work (1) indicated that *Physalospora* thrives well on the apple at such temperatures. After five to seven days the apples were cut through the point of inoculation or in a plane about midway between the stem and calyx and the rot measured at the point of its greatest diameter. Inoculations and measurements were made on the same day. Each experiment was concluded when the largest rots were not over 35 to 40 mm. in diameter.

In order to compare the rate of growth when mycelium and spores were used as the inoculum, an experiment was conducted in which spores were used in one series and mycelium in the other. The results in the two series were essentially the same, although the rot started somewhat more quickly when the mycelium was used.

In 1923, Grimes apples were inoculated on October 16 and November 2, Ben Davis on December 6 and February 8, and Jonathan on December 13.

In 1924, fifty-eight additional cultures were secured and used for inoculations, making in all 155 cultures that were inoculated into Grimes apples on October 9, November 8 and November 14. The number of inoculations made in the two years was about 6,800.

The data obtained from these inoculations are summarized in the graph (Fig. 1). The length of the lines represents the relative diameter of the

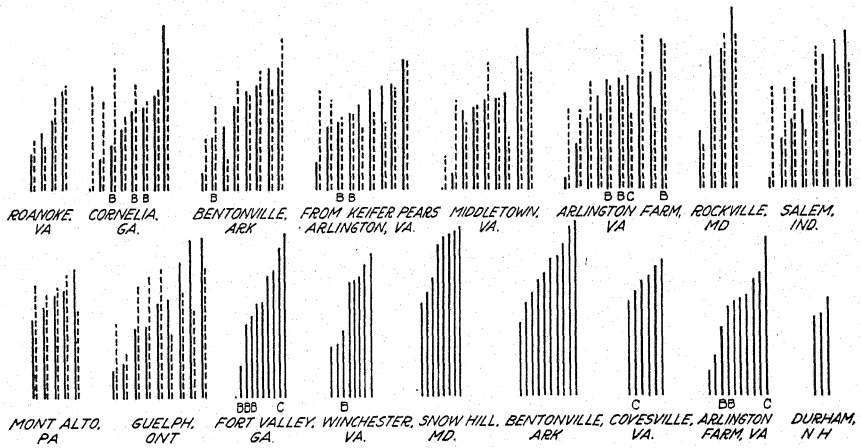

FIG. 1. The broken lines give the relative diameters of the decayed areas in the inoculation experiments conducted in 1923. Each line represents the average of three different experiments involving 8 apples each, or 24 inoculations for each fungus. The solid lines give the relative diameters of the decayed areas in the inoculation experiments conducted in 1924. Each line represents the average of 2 experiments involving 8 apples each, or 16 inoculations for each fungus. Those marked B were *Botryosphaeria ribis* and those marked C were *B. ribis chromogena.*

rot. A study of the data before the averages were made shows that there is a remarkable uniformity in the size of the rots in the apples inoculated with the same culture. It also shows that, in general, the culture that produces either a slow or a rapid decay in one experiment will behave in a similar manner in other experiments. In other words, the differences in the rate of growth of various cultures are fairly constant throughout a series of inoculations on different varieties. One might suppose, if there is a decided variation in the ability to produce rot based on environmental factors, that the cultures from Canada might represent one extreme, those from Georgia another, and that those from Maryland might lie about midway between, but a glance at the graph shows that such was not the case. The cultures from one locality were not materially unlike those from another. On the other hand, the size of the rots produced in a given time by the different cultures from the same locality showed a wide variation. In

most groups the largest rot is at least double in size, and in some cases even treble that of the smallest rot in that particular group. The cultures from one locality varied in the size of the rot produced about as much as those from another. The average diameter of the rots produced within a given time by the different cultures in many of the groups varied from about 12 to 24 mm.

The graph further shows that, with the exception of a few cultures which lost their virulence before the second year, the rot produced in 1924 was comparable in size with that produced by the same fungus when freshly isolated in 1923. The cultures were kept at about 3° C. during the summer of 1924.

The data on the graph indicate that, if a group of cultures from any one locality was large enough, it would represent a series differing from one another in only a slight degree. The range of pathogenicity in the series would be wide, the cultures at one end of the series causing a small rot with an average diameter of 5 mm., those at the other end of the series causing a rot with a diameter of 35 or more mm. A study of the graph shows that one very readily could select a culture from the middle of one of the larger groups and set it apart from those at either end of the group as being sufficiently different to be a distinct strain. This would be even more true if one took for comparison the cultures with lowest and highest rotting capacities. It is possible that, if one isolated only a limited number of cultures, he might have only those representing a low pathogenicity and those representing a high pathogenicity and thus seem to have ample ground for two or more decidedly different strains.

As will be noted, the comparison of the sizes of the rots is made on the basis of the simple diameter. This seemed to be the best method, yet it has its objections. The comparative measure of the actual rot caused by two cultures of fungi in a given time should be based on the volume of tissue decayed. If the rotted portions were exactly similar, their volumes would be to each other as the cubes of their diameters. An example of the difference in the end results between the ratio when based on the simple diameter and when based on the cube of the diameter may be cited in two cultures from Ontario, the diameters of whose rots were 12.7 and 25.3 respectively or in the ratio of about 1 to 2, while the ratio of the volumes would be 2,048 to 16,194 or 1 to 8.

In order to determine if there were any discernible morphological differences in the cultures that could be correlated with their variation in pathogenicity, the cultures were all grown on cornmeal paste and placed in the greenhouse under conditions that favored pycnidial production. By availing ourselves of the discovery of Shear, Stevens, and Wilcox (5), we

had the thrill of getting *Physalospora* to fruit at will. Both the cultures isolated in 1923 and 1924 showed the remarkable efficiency of 100 per cent fruiting. An opportunity was therefore afforded for observing the pycnidia of each culture. No sharp and definite morphological differences were detected, distinguishing the southern from the northern cultures, or the more vigorous rotters from the less vigorous.

One very interesting discovery was made in connection with the work, an account of which is given by the junior author (3). She reported that there is another organism causing a rot of the apple that is so similar to that produced by *Physalospora* that the two are indistinguishable macroscopically. Both the chromogenic and non-chromogenic forms of this species were isolated and found capable of producing a rot on apple. The cultures marked B in the graph are *Botryosphaeria ribis* G. & D., and those marked C are *Botryosphaeria ribis* f. *chromogena* G. & D. A study of the data recorded in the graph leads us to conclude that *Botryosphaeria ribis* and its related form, *Botryosphaeria ribis chromogena,* are both capable of producing a rot in the apple.

SUMMARY

1. In 1923 and again in 1924 *Physalospora malorum* was isolated from rotting apples in 14 different localities from southern Georgia to Guelph, Ontario, and from the Atlantic coastal plain to Arkansas. Groups of about 10 cultures from different fruits were obtained from each locality, making a total of 155 cultures. Inoculations were made in apple fruits in 1923 with fresh cultures, and in 1924 with both fresh and year-old cultures. The total number of inoculations was about 6,800.

2. In many cases the cultures of the black rot fungus from the same locality exhibited a wide variation in the size of the rot produced in a given time when inoculated into the apple. In some groups the average diameter of the largest rot was more than twice that of the smallest. As much variation was found within a group from a certain locality as there was between the groups from different localities.

3. *Botryosphaeria ribis* and *B. ribis chromogena* were isolated from rots resembling black rot and when inoculated into the apple produced a similar rot. Both the chromogenic and the non-chromogenic forms of *Botryosphaeria* exhibit a variation in pathogenicity that is not unlike that of *Physalospora.*

4. The rots produced by *Physalospora* and by the two forms of *Botryosphaeria* were indistinguishable.

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WASHINGTON, D. C.

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THE COTTONY LEAK OF EGGPLANT FRUIT CAUSED BY PYTHIUM APHANIDERMATUM

CHARLES DRECHSLER

In connection with a study of the genus *Pythium* pursued for several years, the writer has found it expedient to test out the pathogenicity of the various types encountered, by inoculating them into certain plant products obtainable on the market. An assortment including, for example, watermelons, cucumbers, squashes, potatoes, and cabbage has been found to provide a suitable variety of host materials, the reactions of which together give a general indication of the virulence of any particular organism tested. Eggplant (*Solanum melongena* L.) fruits were similarly found convenient and have been employed to some extent. They appear subject to attack in varying measure by a considerable number of strains of the type distinguished by smooth oogonia, subspherical sporangia (or "conidia"), and the production of cottony aerial mycelium, that have been assigned largely through custom to *Pythium debaryanum* Hesse. However, these forms were in all cases less energetic in their parasitism than *Pythium aphanidermatum* (Edson) Fitzpatrick. Strains of the latter, whether derived from cucumbers affected with cottony leak,[1] from watermelons affected with buff blossom-end rot, from pea roots affected with root-rot, or from dead female rootknot eelworms, *Caconema radicicola* (Greef) Cobb., uniformly proved very rapid in bringing about destruction of inoculated specimens.

That such parasitism is not confined to experimental material became evident through the receipt from C. D. Sherbakoff of a culture of a fungus isolated from diseased eggplant fruit collected by E. L. Felix in a field near Knoxville, Tennessee, September 18, 1923. According to a communication from Mr. Felix, the fungus in question had been responsible for an estimated loss of one per cent of the crop. It was readily identified as *Pythium aphanidermatum,* exhibiting the characteristic morphology of this species—numerous swollen digitate or lobulate processes that promptly develop into multiple sporangia, and sexual apparatus in considerable abundance, of which the most distinctive element is the intercalary antheridium with its dome-shaped or barrel-shaped protuberant part broadly adnate to the oogonium.

[1] Drechsler, Charles. The cottony leak of cucumbers caused by *Pythium aphanidermatum.* Jour. Agr. Res. 30: 1035–1042. 1925.

On July 20, 1925, a decay of eggplant fruits similar to that resulting from artificial inoculation with *Pythium aphanidermatum* was encountered in a number of fields in the vicinity of Bradentown, Florida. Some diseased specimens were collected and bits of affected tissue planted on cornmeal agar, with the result that 18 pure cultures of *Pythium aphanidermatum* were obtained, each from a separate, individual affected fruit. A close adherence to the morphology represented in isolations of the fungus from other sources permitted the identification of these strains with much certainty. On cucumber fruits they brought about the pronounced softening of tissue and produced the abundance of extramatrical mycelium associated with cottony leak.

The decay of eggplant fruits induced by *Pythium aphanidermatum* is an unusually rapid one, the rapidity being indicated, for example, in Plate V, A and B, representing photographs taken, respectively, 48 hours and 72 hours after inoculation by means of an incision at the blossom end. The destruction of the fruit, which measured 125 mm. in length and 110 mm. in diameter, was complete 78 hours after inoculation, at which time the extramatrical mycelium had become so profuse as to clothe the specimen almost entirely in a cottony weft. Externally the progress of the fungus becomes evident in the disappearance of the normal purple pigment, so that the affected region is distinguished by a tan coloration rather similar to that characteristic of lesions caused by *Phomopsis vexans* (Sacc. & Sydow) Harter. The zonation and surface depression distinctive of the latter parasite is absent however, the epidermis instead generally becoming minutely wrinkled, and rubbing off from the underlying tissue on very slight friction. Internally the advance of the parasite is marked by dark sepia-brown discoloration and pronounced softening in texture of invaded parts. Although, owing probably to the firmness of the outer tissues, affected eggplant fruits are at the beginning somewhat less watery than cucumbers attacked by the same parasite, in the more advanced stages a brownish liquid usually drains off in quantity. The name "cottony leak" applied to the cucumber trouble would seem to be equally appropriate here.

In the fields visited, the occurrence of the disease was confined to limited areas where poor drainage was keeping the soil in a soggy condition, especially in the furrows between the rows. All of the infected fruits were found resting with the blossom-end in contact with the moist ground, to which they were cemented, as it were, by a heavy weft of mycelium, so that, upon lifting the fruit, masses of soil were found adhering in a moldy cake. Infection in all cases appeared to have begun at the blossom end and progressed toward the stem end. No high-hanging fruits were found affected with cottony leak in any portion of any of the fields, wet or dry.

The value of adequate drainage as a method of control is clearly indicated. The season during which eggplants mature in Florida would seem generally too dry to permit the trouble to become a serious one. In the absence of pertinent information, it would seem, however, that in the more northern latitudes where rains in July, August, and September are frequently sufficient to keep the ground saturated several days at a time, losses from cottony leak might well assume proportions deserving of consideration. Owing to a certain degree of resemblance, particularly in external discoloration, of the affected fruit with fruit attacked by *Phomopsis vexans*, it is highly probable that the disease, where observed, has generally been attributed to the latter parasite. Several observers have informed the writer that instances of fruit-rot have come to their attention in which no indications of pycnidia were to be found on large well-developed lesions.

It may not be amiss to add that a species of *Pythium* of the "debaryanum" type was isolated in a single case of eggplant fruit-rot, which was found capable of reproducing the disease when inoculated into the healthy fruit. Its effect on the tissues was very similar to that of *Pythium aphanidermatum*, but the rate of destruction was approximately only one-half as rapid, and the production of aerial mycelium relatively meager. Undoubtedly, as in the similar disease of cucumbers, the capacity of the extramatrical mycelium abundantly produced by *Pythium aphanidermatum* to effect penetration through the unwounded external tissues of the host, rather than a superior degree of virulence, is responsible for the preponderance of this species in naturally infected eggplant fruit.

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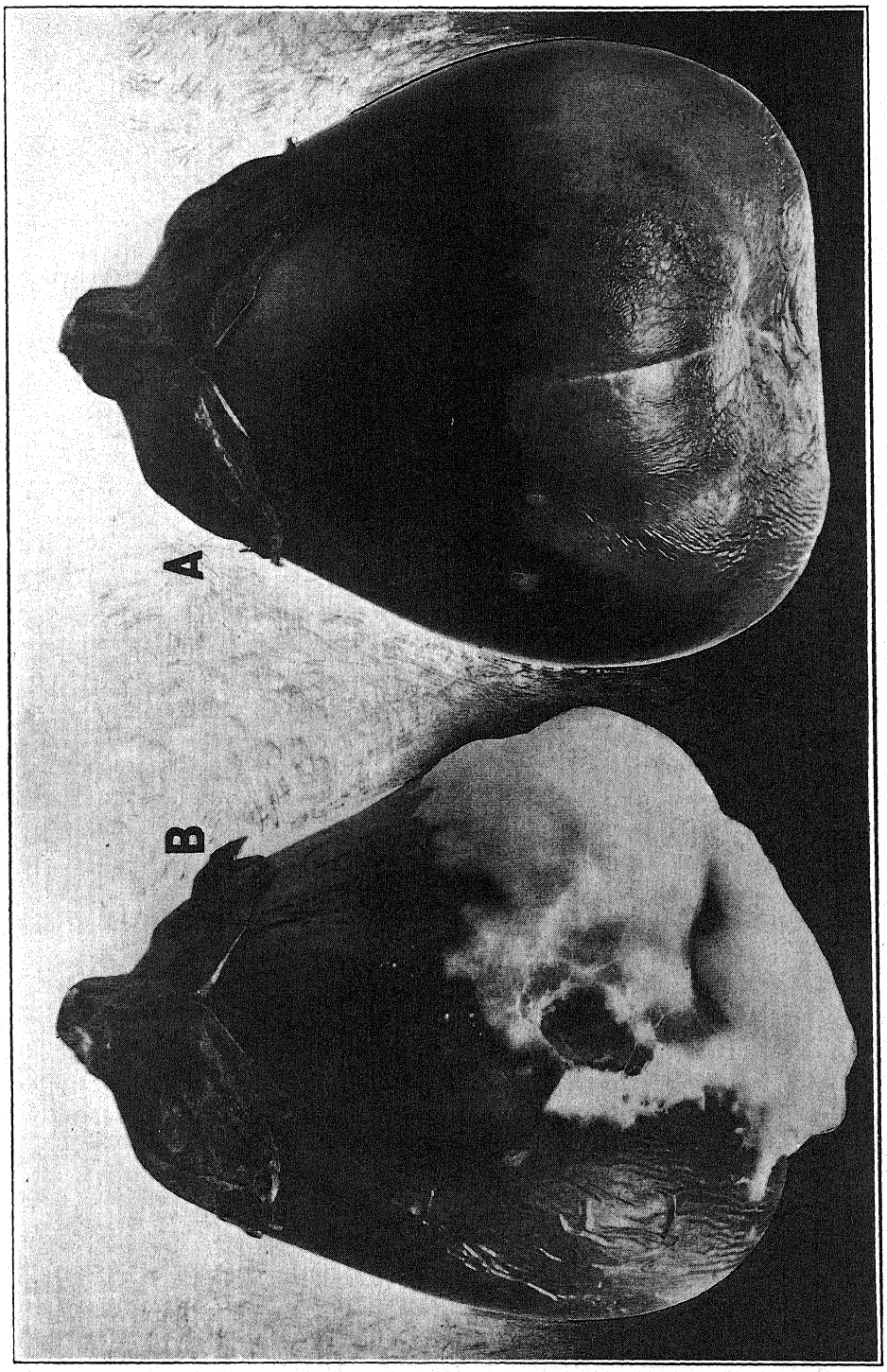
EXPLANATION OF PLATE V

FIG. A. Eggplant fruit 48 hours after inoculation by incision at blossom end with pure culture of *Pythium aphanidermatum*; lower distal half of fruit tan-colored externally, and epidermis folded in minute wrinkles. × ¾.

FIG. B. Same fruit as in A, but 24 hours later; exterior entirely tan-colored, except a small proximal region at upper left-hand corner of picture; copious development of extramatrical mycelium from earliest invaded portion. × ¾.


A
B

FOOT-ROT OF LILIUM CANDIDUM AND LILIUM PYRENAICUM CAUSED BY PHYTOPHTHORA CACTORUM

CHARLES DRECHSLER

On May 2, 1925, a diseased Madonna lily plant, *Lilium candidum* L., from an experimental plot maintained by H. E. Allanson at Tacoma Park, Md., in the immediate vicinity of the District of Columbia, was presented to the writer for examination. The specimen was submitted as illustrative of a trouble responsible for the loss of individuals of this species that had occurred from time to time during the preceding weeks. Absence of any pronounced premonitory symptoms in the foliage or in the aerial portion of the stem was reported to be a characteristic feature, the disease generally becoming apparent rather suddenly in a falling over of the entire structure above the ground. Once felled in this way, the affected individuals soon withered and died.

On examination of the specimen, the seat of the malady was readily located in the subterranean part of the stem, which, beginning somewhat abruptly at a level approximately with the ground line, was shrunken to a part of its normal diameter. The parenchyma external to the strands of conducting elements was completely collapsed. Both in this tissue and in the central pith, the microscope revealed an abundance of non-septate intercellular mycelium provided with diverticulate protuberances. Pieces of affected tissue from the shrunken region as well as from the marginal zone bordering on the healthy aerial part of the stem were placed on corn-meal agar plates. From each of the pieces the same fungus was thus isolated in pure culture—a species of *Phytophthora* producing little aerial mycelium, but an abundance of sporangia and oospores.

On May 28, 1925, a specimen of *Lilium pyrenaicum* Gouan was submitted, which had been obtained from the same plot as the Madonna lily, and which had succumbed evidently to the same disease. Except that the healthy portion of the stem extended somewhat below the ground line, the macroscopic as well as the microscopic findings were entirely similar. And from all platings of diseased tissue the same species of *Phytophthora* was again isolated.

The parasite from the two species of lily was grown in Petri dishes on various culture media, parallel with species of *Phytophthora* with which its morphology suggested a possible relationship. A high degree of similarity

with respect to rate of growth and general appearance of thallus to the species most commonly known under the binomial *Phytophthora cactorum* (Lebert & Cohn) Schroeter at once became obvious. This similarity was equally pronounced with reference to microscopical features. Measurements of the diameters of 25 oospores gave an average of 26.4 μ for the fungus isolated from *Lilium candidum,* and 27.1 μ for the form from *Lilium pyrenaicum*; while an equal number of measurements of length and breadth of conidia gave as averages for the two strains 35.7 x 27.3 μ and 34.9 x 27.9 μ respectively. These figures show a satisfactory agreement with the averages obtained from comparable measurements of the several strains of *P. cactorum,* including one obtained from the Centralbureau voor Schimmelculturen at Baarn, Holland, another isolated from rhubarb affected with foot-rot by W. S. Beach, and four strains isolated from diseased apples originating in different localities in the United States. The lily fungus is therefore referred to *Phytophthora cactorum,* thus adding two additional hosts to the list of plants attacked by this parasite.

In this connection, mention may be made of a serious disease of the Easter lily, *Lilium longiflorum* Thunb., reported by H. H. Whetzel[1] as prevalent in Bermuda in 1921, which was designated as "stump rot" and attributed to an unnamed species of *Phytophthora.* According to the account of the Bermuda disease:

> "The pathogene is harbored in the soil. Spores produced at the surface of the soil are splashed by falling rain into the crowns of the plants. Here a rot is rapidly set up which may destroy the entire stalk down through the bulb. Or only the growing tip may be killed leaving a stump with a rosette of leaves. . . . Attacks shortly before flowering are common. In these cases the tip of the stalk alone is usually killed."

The symptoms described evidently differ widely from those distinctive of the trouble that prevailed at Tacoma Park in the spring of 1925, the latter being perhaps most similar to those associated with foot-rot of tomatoes, attributable to *P. cryptogea* Peth. & Laff.[2] Future observations may determine the extent to which the relatively dry season during which the American disease developed may have affected its severity or modified its character. It is scarcely likely that the difference in specific identity of the congeneric hosts could have provided a factor of much importance, since the relationship between the Easter lily and the Madonna lily is usually

[1] Whetzel, H. H. Report of the pathologist for the period June 10 to December 31, 1921. Bermuda Repts. Bd. & Dept. Agric. 1921: 30–64. 1922.

[2] Pethybridge, G. H. and H. A. Lafferty. A disease of tomato and other plants caused by a new species of *Phytophthora.* Sci. Proc. Royal Dublin Soc. 15: 487–505. 1919.

regarded as a close one—considerably closer than that between the Madonna lily and *Lilium pyrenaicum*. It is not impossible that lilies, like certain other hosts, may ultimately be found subject to attack by more than one species of *Phytophthora*, each responsible for symptoms revealing a measure of correspondence to, or of distinctiveness from, those induced by the others.

BUREAU OF PLANT INDUSTRY,
U. S. DEPT. OF AGRICULTURE,
WASHINGTON, D. C.

OVERWINTERING OF BACTERIUM PRUNI

H. W. ANDERSON

During the past five years the writer has frequently attempted to isolate *Bacterium pruni* E. F. S. from cankers on peach twigs and from the buds during the dormant period, since he felt that Rolfs[1] had presented adequate proof that the bacteria wintered in these situations. Repeated failures in these attempts, together with the observations on the scarcity of cankers in many badly infected peach orchards, led to the conclusion that possibly other methods of overwintering might prevail under Illinois conditions. During the past winter, therefore, many isolations were attempted from all sources which reasonably could be expected to harbor the bacteria.

As a result of these isolations it has been established that the bacteria are able to overwinter in the dead leaves of the peach. No bacteria resembling *B. pruni* could be isolated from buds, even when these buds were obtained from twigs which had borne heavily infected leaves the previous summer. Cankered twigs were obtained throughout the dormant period from many localities in Illinois and other states, and several hundred attempts were made to isolate *B. pruni* from them, with uniformly negative results. No attempt will be made in the present article to present the negative results, although they are considered of value, and, if confirmed by another season's work, will be published in detail.

Rolfs[2] states that "cankers on the twigs and limbs are the principal sources of infection in the spring" and that "the tissues of the bud may likewise become invaded and are often responsible for the early spring infection." In regard to overwintering of the bacteria in leaves, he states: "Old leaves on the ground, if well protected from sun and air, may possibly harbor the organism for some time, but it is doubtful whether they constitute an important source of infection." He was able to isolate the organism in the spring from inoculated twigs which had died during the winter. He does not state that he ever isolated the bacteria from naturally-formed cankers after the first year. He isolated bacteria from the buds in August but gives no proof that they live there over winter. He attempted to make isolations from dead leaves but was unsuccessful except when they were left between blotters in the laboratory from October to June of the following year.

[1] Rolfs, F. M., A bacterial disease of stone fruits. N. Y. (Cornell) Agr. Exp. Sta. Mem. 8: 381–436. 1915.

[2] *Ibid.*, p. 413–415.

Roberts[3] states: "The twig lesions are of importance, however, in that in these the causal organism passes the winter."

While Lewis[4] states that the cankers on plums remain in evidence for several years, he made no isolations except from fresh cankers.

No other definite statements as to the method of overwintering of the bacterium on the peach are found in the various publications on this disease. However, it seems to be generally accepted by pathologists and horticulturists that initial infection results from the overwintering cankers.

OVERWINTERING IN LEAVES

In 1922, isolations were made early in the winter from leaves from beneath peach trees. It was extremely difficult, however, to find entire leaves in the spring, and, due to lack of experience, no spots could be identified with certainty on the small fragments which were found. Leaves placed in wire screens were completely disintegrated before spring.

During the winter of 1924–25, further attempts were made to secure cultures from leaves. Work was started as soon as the leaves began to fall, and isolations were made at least once a week until December. From the latter part of December until the opening of the buds in the spring, isolations were attempted only at intervals of two or three weeks.

Weather conditions in 1924–25 were very unfavorable for the overwintering of the bacteria. October was extremely dry, allowing the foliage to become thoroughly dried. In November several rains soaked the foliage, and continued mild weather favored decay. In December wet weather prevailed in the early part of the month, with temperatures as high as 60° F. A very heavy sleet storm on December 17 and 18 covered all exposed objects with ice. On the 20th the temperature fell to below zero F., and zero temperatures prevailed until the 30th. On the 28th, the temperature went to 20° below zero. The ice glaze continued throughout January, but warm weather prevailed in February and March. One would expect that only a few of the bacteria could survive these extreme conditions.

Successful isolations were made on January 16, February 25, March 5, and April 2, from leaves from the orchard at Urbana, and on March 10 from leaves from an orchard in Johnson County in southern Illinois. In contrast to the abundance of bacteria in platings from the leaves, platings from twigs and buds showed only a few yeasts or other fungi.

INOCULATION EXPERIMENTS

On June 5, 1925, four cultures were used to inoculate leaves of Elberta peach in the University orchard, Urbana. The first two were isolated from

[3] Roberts, J. W., Control of peach bacterial spot in southern orchards. U. S. Dept. Agr. Bul. 543. 1917.

[4] Lewis, I. M., A bacterial canker of plum twigs. Amer. Micros. Soc., Trans. 31: 145–149. 1912.

green leaves from Tunnel Hill, Illinois, on June 1, 1925, while the other two were isolated on March 5 from dead leaves collected at Urbana. The first cultures, therefore, represent recent isolations from living leaves, while the second series represents bacteria overwintering on old leaves and kept on artificial media for three months. Weather conditions during April, May, and the first week in June had been extremely dry, with practically no rain. Consequently no natural infection had taken place in the orchard.

On June 19 there was no trace of natural infection of other trees in the orchard, but abundant infection was observed on the leaves of two twigs inoculated with the recently isolated cultures, while lighter infection was noted on the leaves of two twigs inoculated with one of the cultures from dead leaves. On June 21, infection was observed on the leaves of all the inoculated twigs. The position of the spots and the general nature of the infection indicated strongly that the infection resulted from the artificial inoculation. No spots were found on the checks nor any other part of the tree, and none had appeared by July 15.

On June 25, a few cases of natural infection were found on other trees in this orchard. These were extremely scattered and only one or two infected leaves could be found on each tree. Only five trees in more than a hundred examined showed these scattered infections.

Isolations were made on June 19 and June 20 from several of the leaves from both series, and in every case typical pure cultures of *B. pruni* were obtained. This completed the proof of the identity of the organisms isolated from the dead leaves. Other inoculations both on leaves and young shoots were subsequently made with the same results.

SUMMARY

The methods of overwintering of *B. pruni* have been studied over a period of five years.

While it seems to have been established by others that the bacteria are capable of overwintering in cankers, the evidence from a study of the commercial peach orchards of Illinois would indicate that this is not the common source of initial infection.

No great difficulty has been experienced in isolating the bacteria from dead leaves in the spring, and consequently it would seem that these bacteria might be responsible in some cases for the initial infection.

Just how inoculation is brought about is not known, but, since the bacteria occur in rather large, gelatinous masses in which they are protected from desiccation, it may be assumed that these are blown as dust particles or in fragments of leaves to the new foliage and, when moistened, readily cause infection.

UNIVERSITY OF ILLINOIS,
URBANA, ILLINOIS.

BOOK REVIEWS

Éléments de Pathologie Végétale, Appliquée a l'Agronomie et a la Sylviculture. By E. Marchal. 312 pages, 148 figures. Jules Duculot, Editeur. Gembloux. 1925.

The science of plant pathology is rapidly growing through the activity of research centers in Europe, and, as the author properly remarks, also through the marvelous organization of phytopathology in the United States.

On account of the rapid accumulation of new literature and because of the scarcity in the French language of books covering the field, the author has compiled a volume embracing the diseases of farm crops, fruits, and forest trees in Belgium. A career of thirty years in that country as plant pathologist to the State has given Professor Marchal full opportunity to gather material for the book. The author does not neglect mentioning the important farm and forest crop diseases in other countries; he pays some attention to the more outstanding work done within recent years in the United States, and likewise writes on the diseases of cultivated plants in the tropics of the Belgian Congo.

It is always with interest that a new book on plant diseases is examined. A number of questions arise in regard to the organization, method of approach, point of view, emphasis, and disposition of controversial topics; consequently one is led promptly to see what disposition has been made of these matters.

The author recognizes that phytopathology, in the larger sense, deals with all phases of diseases of plants. These diseases, he states, are on the one hand due to parasites (parasitic diseases), and on the other are produced through the action of unfavorable factors of environment (non-parasitic diseases). Unfortunately, the author has failed to take advantage of a more accurate usage, as have a number of American pathologists, in that he uses "physiologic diseases" synonymously with non-parasitic diseases. This type of oversight, however, is not the rule in this book.

Agents of parasitic diseases belong, he says, to the plant or to the animal kingdom. In many countries plant pathology is restricted to plant diseases of vegetable and non-parasitic origin. Thus, feeling that the science is a phase of botany, he leaves the parasitic diseases of animal origin to the entomologists. It is in this more restricted sense that he uses the term plant pathology.

This book is another example of a work in which the subject is approached through the field of mycology and bacteriology. The table of contents is a clear index to his manner of approach to the field of pathology. The point of view is obviously one of economic mycology. It is true that in the field of plant pathology he recognizes such phases as symptomatology, etiology, hygiene, prophylaxis and therapeutics. No apparent effort is made to explain symptomatic features through a study of infection; no attempt is made to link etiology with control; in other words, the book deals only to a minimum degree with analytical plant pathology. The author seems rather more interested in descriptive pathology, explaining for each disease discussed something of its appearance (diagnosis), brief life history points where a parasite is the cause, economic aspects, striking environmental factors, and control. And further, it seems probable that Professor Marchal attempts through this book to stimulate particular interest in plant diseases. He mildly laments that hitherto phytopathologic questions have not aroused the interest in Belgium which they merit. He finds consolation in part, however, through the fact that there have been no outbreaks of plant diseases in Belgium sufficiently serious to

arouse public opinion. Attention is called to the utilitarian character of plant pathology, public and professional recognition of which is emphasized through phytopathological inspection service, extension service, conferences, congresses, the press and scientific periodicals.

The author devotes a chapter to parasitism and parasites. He approaches the concept of parasitism through a brief excursion into the field of plant physiology. He defines virulence as that property which distinguishes parasites from saprophytes in their ability to attack living tissues. This characteristic lies, he holds, in the ability which certain organisms have of secreting substances capable of breaking down the means of defense of the host. These substances he classifies as enzymes and substances comparable to the toxins of pathogenic bacteria. Virulence may be fixed (constant) or variable; in obligate (absolute) parasites this property is constant, while in faculative forms it is variable. Through nutritional means, organisms may be induced to exalt their virulence or they may suffer loss of virulence. Parasites, depending on their restrictive host-habits, are termed polyphagus and isophagus. Immunity results, the author explains, when resistance (host defense) is victorious over virulence to the extent that the host is refractory to the infection. It is pointed out that in plants the subject of immunity has been largely neglected. Mechanisms comparable to phagocytes do not seem to exist, and while hyphae are observed to be englobed and digested, yet nothing similar to antitoxins is implied. Host-defense is classified as mechanical (anatomical) and chemical (physiological).

The specific plan of the book includes Part I, dealing with parasitic diseases of vegetable origin, and Part II, "Les Maladies physiologiques."

The general introductory chapter deals with (a) parasitism and parasites, and (b) means of control, including brief, general discussion of legislative measures, cultural measures, prophylactic and therapeutic measures. Discussion of specific diseases begins with chapter two, in which about eight pages are devoted to bacterial diseases such as blackleg and scab of potato, fire blight, olive knot, and bacterioses of beet, cabbage and tomato. In the section on myxomycetous diseases, less than two pages are devoted to club root, with probably one hundred and fifty words on powdery scab of potatoes. Fungous diseases occupy more than half the book. Two paragraphs are given over to a general statement on Phycomycetes, followed by one-half page on Peronosporales. Less than one-half page is devoted to *Pythium de Baryanum*, while *Pythophthora infestans* is treated less sparingly (six pages, more or less). Due proportion is given over to ascomycetous diseases.

Apple scab is described in the following order: symptoms, etiology (largely morphology of the fungus), nature of losses, environmental influences, and means of control. Destruction of old leaves, fruits and twigs is advised. It is also recommended that affected twigs be cut off when pruning. It is advised that the trees be sprayed with strong Bordeaux mixture before buds open, again at the petal fall, and once again fifteen to twenty days later. In cases where one wishes to give the minimum treatment, the dormant spray may be omitted, and only the application after pollination should be made. The quantities of spray mixture for various sizes of trees are specifically given (*e.g.*, 25–30 liters for a tree 8 meters crown diameter; for a hectare, 1,500–3,000 liters). Smuts, rusts, polypores and Fungi Imperfecti are more or less proportionately included. Just why the author skips over the *Fusarium* problem is not clear. Loose smut of wheat is evidently not serious in Belgium, since the author passes over the seed treatment by mere reference; the method to be used is not described. He advises, for Belgian conditions, the selection of healthy plants for seed, and, where safer methods against infection are desired, it is recommended that the heads be protected by enclosing them in gauze.

In a section dealing with black stem rust of cereals, the work of Stakman and his colleagues on physiologic forms is not wholly overlooked, yet full discussion is lacking. Practically nothing is said regarding large scale eradication of barberries; he merely advises the destruction of aecial hosts when their immediate presence manifestly influences the abundance of rust.

Filterable virus diseases are treated in a chapter set as an appendix to the study of parasitic diseases. The seven or eight pages devoted to these diseases are interestingly written although somewhat lacking in completeness. The characteristic feature of filterable virus diseases is stated: a group of diseases presenting the remarkable peculiarity of being transmissible from a diseased to a healthy plant by inoculation or graft, without one's being able to detect in the infected tissues the presence of a causal organism. He hardly accepts the ultramicroscopic-bacteria theory, although he does not fully reject the living organism hypothesis. Mosaic and other degeneration diseases of potato, tomato, and sugar-cane are specifically discussed, while mere mention is made of these diseases affecting other crops.

The second part, which treats of non-parasitic diseases, considers the soil, its physical properties, chemical composition, effect of lack of water, excess of water, and nutritive features. Light, cold, rain, snow, hail, wind, lightning—all are given some attention in so far as they are concerned with the subject at hand. Wounds and regeneration of organs are briefly touched.

A key of some twenty pages for the determination, by external characters, of the more common diseases is probably commendable as an incentive for someone else to do better. One is only reminded of the necessity of much further study on symptomatology as such, before valuable analytical keys can be constructed.

The book is of value as an index to crop diseases in Belgium. It seems doubtful that it can be useful as a text: it is too superficial for class use, and principles are largely obscured or not mentioned. For the average student, references to literature are most valuable; yet the author has cited no references whatever.

L. R. HESLER.

Winter Botany. A companion volume to the author's *Plant Materials of Decorative Gardening.* By Wm. Trelease. Second Edition, Revised. XLII+396 pages, 328 figures. Published by the Author, Urbana, Ill. 1925.

The second edition of this handy little volume bears the same title as the first, published in 1918, and remains, as stated in the introduction, essentially unchanged except for a few corrections and the addition of two genera (*Casuarina*, p. 9; and *Pyrularia*, p. 45).

The text deals with a total of about 1,100 species and varieties belonging to 328 genera in 94 families. The arrangement of families is according to the system of Engler. This is a desirable feature, since it avoids the confusion of being at variance with the standard floras and manuals of the country. From the standpoints of taxonomy and nomenclature, the work is conservative, and the user does not need to grope around among unfamiliar generic names nor puzzle over hair-split species.

The volume is profusely illustrated with marginal figures showing the essential details of leaf scar, buds, pith, and other important characters. Since descriptions are wanting, except for genera, these illustrations are indispensable for the correct identification of species, and because of their uniform excellence they constitute an outstanding feature of the book. References to literature on winter characters of individual species are cited after most of the generic descriptions, and in many cases valuable

observations and comments are added. A brief bibliography and a comprehensive glossary are supplied at the end of the text.

Bracket keys are used throughout, and it is hoped that their employment commended itself to the author rather from the necessity of the small pages than from choice. The key to genera runs through 30 closely printed pages with over 468 contrasting dichotomies. A bracket key of such length, compelled as it is, because of the nature of the material, to use over and over again identically worded contrasts such as "leaf scars raised—leaf scars low; twigs slender—twigs stout; buds superposed—buds not superposed," is inherently confusing and is difficult even for the experienced to manipulate.

The reviewer cannot help but feel that the value of the book, especially for the use of the non-professional botanist, would have been greatly enhanced if the monotonously long bracket key to genera could by some device have been broken up into a number of distant major divisions, or, better still, if an indented key had been constructed instead. If mechanical difficulties alone necessitated the use of a distinctly inferior type of key, it seems that a better plan would have been to reduce the scope of the work and deal with the material covered in separate parts or volumes, one for each of the two or three major floristic regions into which the 1,100 species fall. The attempt to treat such a floristically heterogenous region as the United States and adjoining Canada in a single handbook must inevitably result either in serious abridgement of the means and methods of classification, or, on the other hand, in a cumbersome product.

If it is maintained that the work deals primarily with decorative species, removed from their respective floristic regions, the fact nevertheless remains that numerous forms are included which thrive out-of-doors only in the extreme South, Southwest, or in California, and, if cultivated at all in the colder parts of the country, they are grown under glass, in which case flowers and fruits are frequently available in winter.

C. O. ROSENDAHL,
DEPARTMENT OF BOTANY,
UNIVERSITY OF MINNESOTA

ABSTRACTS OF PAPERS PRESENTED AT THE SEVENTEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, KANSAS CITY, MO., DECEMBER 29 TO 31, 1925

The field survey as a basis for the phenological interpretation of the plant disease epidemic. L. R. TEHON.

From quantitative records accumulated by the State Natural History Survey in its plant disease survey of Illinois, those of three selected diseases—potato late-blight, wheat leaf-rust, and apple bitter-rot, are briefly tabulated and the prevalence and intensity of each correlated with temperature and rainfall by means of climographs.

From Martin's data on late-blight we may construct climographs showing July temperature and rainfall combinations productive of "severe blight," "light blight," and "no blight." Normal July temperature-rainfall combinations for certain localities in the northern and southwestern Illinois potato districts occur outside of the limits for blight. The interpretation that both of the Illinois potato regions have temperature-rainfall combinations not favoring late-blight development is substantiated by field records, blight never having been found in the southwest and only rarely in the north.

Similar climographs, based upon field data from Illinois, indicate correlations between the intensity of leaf-rust and bitter-rot infection and temperature-rainfall combinations. With adequate field data at hand, this method will measure phenological influences with an accuracy permitting considerable practical use.

The bioluminescence of Omphalia flavida, a leaf-spot fungus. A. H. R. BULLER and T. C. VANTERPOOL.

Maublanc and Rangel at Rio de Janeiro found the fruit-bodies of *Stilbum flavidum* Cke. and an *Omphalia* on spotted leaves of *Eriobotrya japonica*, etc., kept very moist; and they concluded that the *Omphalia* was the perfect stage of the fungus. S. F. Ashby, in a recent number of the Kew Bulletin, has proved this connection by the pure culture method, his isolations having been made from *Stilbum* heads produced on spotted coffee leaves. The senior author, using sub-cultures of Ashby's material, discovered that the mycelium is luminous in the dark. In further work the authors have obtained typical leaf-spots on wounded and unwounded *Bryophyllum* leaves and on wounded *Oleander* and *Ficus* leaves. The leaf spots were faintly luminous in the dark. About ten days after inoculation typical saffron-yellow so-called *Stilbum* fruit-bodies began to develop at the surface of the infected leaf-areas; and, after another ten days, at the edges of the same areas, a few typical *Omphalia flavida* fruit-bodies made their appearance. The mycelium is luminous on bread, oat-meal agar, and malt agar. The fruiting structures of both kinds give out no light whatever. No other parasitic fungus is known to produce luminous leaf-spots. Luminous leaves have been produced artificially for the first time.

Organic mercury compounds for the control of loose smuts of wheat and barley and barley stripe. I. L. CONNERS.

One-hour soak in Uspulun (0.25 per cent solution), Germisan (0.25 per cent solution) or Semesan (0.3 per cent solution) at room temperature, gave practical control of stripe (*Helminthosporium gramineum*) in Minsturdi barley. Germisan seemed the most effective. Eight per cent of stripe developed in the check plot. The modified hot-water treatment failed to control (3.5 per cent).

Loose smut in Kota wheat and Junior (hulless) barley was not controlled by the above solutions of organic mercury compounds at room temperature. Partial control, especially of loose smut of barley, was obtained with the solutions at 45° C. A presoak in water for one hour increased the effectiveness. Germisan was most effective, but it injured the germination of the barley seed. The modified hot-water treatment eliminated the smuts. The check plots showed over 20 per cent in the wheat and nearly 10 per cent in the barley.

Preliminary experiments on the control of leaf and stem rusts of wheat by sulphur dust. D. L. BAILEY and F. J. GREANEY.

Two series of fortieth-acre plots of Marquis wheat at Winnipeg were dusted fortnightly, weekly, semi-weekly, and tri-weekly with sulphur dust. The rate of application of sulphur was 15 lbs. per acre in one series and in the second series 30 lbs. per acre. The plots dusted once every two weeks were rusted as heavily as the checks. One application per week had some influence, and two applications per week had a very marked influence in controlling rust and increasing the yield. Three applications per week at 15 lbs. per acre practically controlled both leaf and stem rust, and the increase in yield was 34 bu. per acre as compared with the average of the check plots. One plot was dusted at the rate of 15 lbs. per acre when rain seemed imminent (7 applications). The percentage infection and yield in this plot were about the same as in the plots receiving two applications per week or a total of 17 applications.

Second report on dusting for cereal rusts. C. V. KIGHTLINGER and H. H. WHETZEL.

The second season's results in dusting with sulfur for the control of rusts on wheat and oats confirms the indications of the preliminary experiments of 1924. They show that proper applications of dusting sulfur will control these diseases to a significant degree.

The total increase on twenty rod-square plots of winter wheat affected with leaf rust only, was 18.5 per cent or 6.6 bushels per acre. The maximum increase on any individual plot was 47.2 per cent or 17.1 bushels per acre, while the minimum increase was 6.8 per cent or 2.7 bushels. The dusted plots were arranged checkerboard fashion, with 20 checks of the same size. Infection was reduced on the average, 48.2 per cent.

The total yield on twenty plots of 2 square rods each of oats, affected with both leaf and stem rust, was increased 19.6 per cent or 8.6 bushels per acre. The maximum yield on any individual plot was increased 35.1 per cent or 19.6 bushels per acre, while the minimum increase was 6.6 per cent or 2.6 bushels. The dusted plots alternated in checkerboard fashion with 20 equal sized checks. Infection was reduced, on the average, 37.9 per cent.

There is some indication that excessive applications of sulfur dust may cause decreased yields in wheat.

Effect of sulfur dust on the development of black stem rust of wheat in a natural epidemic. E. B. LAMBERT and E. C. STAKMAN.

Wheat was dusted in several localities in Minnesota in 1925 with precipitated sulfur at the rate of about thirty pounds per acre, to determine the effect on stem rust. A heavy natural epidemic obtained in most instances. The results support Kightlinger's statements that sulfur dust controls rust under certain conditions. Time of application is a most critical factor. In some experiments, one application virtually controlled rust in spite of a heavy natural epidemic. In others, five applications were practically ineffective, although the first application was made in advance of appreciable rust in the field. Apparently the sulfur is not effective very long after its application. Evi-

dently it must be applied just before a spore shower. It does not seem to inhibit development of uredinia after plants are once infected. In the hard red spring-wheat region, in years when there is a heavy epidemic, it would be necessary to begin dusting when the grain is in flower, or earlier, and continue until the hard dough stage, thus necessitating five or six applications. No two seasons are alike, and it is doubtful whether the proper time could be predicted with even approximate accuracy in any given season. (Cooperative investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Stations.)

Sweet corn seed treatment in 1925. C. S. REDDY, J. R. HOLBERT, and A. T. ERWIN.

All treatments were made at the same time at one place. Liquid treatments were for 1½ hours at 25° C. Yield data on which the following table is based were taken when the corn was in the canning stage and include only corn prime for canning.

Treatment	Location	Percentage increase or decrease in yield from seed of						
		Country Gentleman			Canadian Evergreen		Golden Delicious	All lots
		Nearly disease-free	Diplodia infected	Gibberella infected	Nearly disease-free	Diseased	Diseased	
Water-soaked	Ill.	+ 0.8	− 5.1	+ 3.8	− 1.7	−14.2	+ 1.3	− 2.7
	Iowa	− 7.1	+25.3	+ 9.2	− 4.2	− 6.7	−15.3	− 1.9
Semesan 0.4% (soak)	Ill.	+ 3.6	+49.0[a]	+25.5[a]	+ 7.8	+ 3.9	+14.5	+15.0
	Iowa	− 1.6	+38.0	+ 1.5	− 4.2	+ 2.0	+25.8	+ 5.4
Semesan Jr. (dust)	Ill.	+ 6.3	+44.7[a]	+23.6	+ 7.8	− 8.4	+ 9.2	+11.9
	Iowa	− 1.6	+56.7[a]	+ 8.2	−15.0	+ 2.0	+21.8	+ 3.7
Uspulun 0.4% (soak)	Ill.	− 1.2	+54.1[a]	+24.8	+11.5	+ 2.0	+33.6	+17.5
	Iowa	-12.0	+30.4	+ 4.1	+ 4.2	+ 0.4	+38.7	+ 4.9
Bayer Dust	Ill.	− 4.3	+29.9	+19.1	− 4.5	− 2.5	+ 5.3	+ 5.0
	Iowa	− 7.1	+59.5[a]	+ 7.7	− 0.8	+ 2.4	+21.7	+ 7.5
Corona 620 0.25% (soak)	Ill.	− 5.5	+33.8[a]	+14.0	0.0	−13.3	+ 4.6	+ 3.5
	Iowa	− 9.3	+26.6	+ 4.6	− 6.7	−12.2	+35.5	+ 1.0
Corona 640 S (dust)	Ill.	− 3.6	+30.5	+ 3.8	− 1.7	− 1.5	+ 1.3	+ 3.4
	Iowa	− 7.1	+36.7	+12.8	−14.2	− 4.7	+12.1	+ 1.1

[a] Odds 30 : 1 or greater, others less.

(Investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, with the Bloomington (Illinois) Canning Company, and the Iowa Agricultural Experiment Station, cooperating.)

The dilution method as a means for making certain quantitative studies of viruses. H. H. MCKINNEY and R. W. WEBB.

Although observations have indicated that mosaic viruses increase in plants, this assumption lacks definite proof; and, since questions have arisen concerning the supposed cultivation of a virus *in vitro*, it seemed advisable to test the first assumption experimentally.

Eight consecutive dilution experiments with tobacco mosaic from a single plant were conducted under greenhouse conditions which insured against accidental infection. Since our studies on tobacco mosaic show that infection occurs from soil, this source as well as others was guarded closely.

All dilutions of the virus were made in sterile, distilled water. By inoculating the plants of a given experiment with diluted virus from a mosaic plant resulting from inoculations with highly diluted virus in the preceding experiment, mosaic was obtained in the eighth experiment in practically undiminished amounts from virus which had passed through eight plants and which had been diluted in water equivalent to 10^{-33}. A single water dilution of this magnitude being far too great to produce mosaic, it is evident that the quantity of virus increased in the plants. The mosaic mottling was as illustrated by Iwanowski. All uninoculated controls remained healthy.

These and chemical fractionation experiments indicate that the dilution method offers an opportunity for making certain quantitative studies on the viruses.

(Cooperative investigations between the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.)

New virus disease of tobacco and related plants. JAMES JOHNSON.

Ordinarily only one virus disease of tobacco (*Nicotiana tabacum*) has been recognized in literature, namely, "tobacco mosaic." Two (or three) other viruses obtained from apparently healthy potatoes have been recently described. In addition to these, at least six others may affect tobacco and related plants. Descriptions of four of these have already been submitted to PHYTOPATHOLOGY for publication. These viruses are specific as indicated by symtomatology, differential host range, and properties. Certain combinations of these viruses may produce quite a different set of symptoms and in general appear to be more malignant than when each virus acts alone. Thus far, no evidence has been secured showing that the viruses in question can be modified fundamentally by passage through differential hosts. On account of the frequent use of "tobacco mosaic" in fundamental researches on the filterable virus problem, it seems important that the specific virus used in many of these studies should be more thoroughly described. Generally it will suffice to describe the symptoms, if any, secured on four or five well selected species together with the resistance to ageing *in vitro*. In other cases, however, additional details may need to be given in order to make a satisfactory description.

The destruction of the chloroplasts in tomato mosaic. HELEN SOROKIN.

In the green areas of living tomato leaves affected with mosaic many of the chloroplasts are still normal. As the disease progresses, the chloroplasts disintegrate. In the early stages there appear inside of the chloroplasts refractive, crystal-like bodies which may be either stationary or actively motile. Next, a blister-like swelling, which gradually increases in size, appears on one side of the chloroplast. The chloroplast gradually becomes smaller as the blister-like sphere increases in size. Finally the

chloroplast disappears entirely, and one or several of the crystal-like bodies can be seen inside of the spheres. Here they are practically always actively motile. The spheres are almost transparent, but can be seen readily after staining with very dilute gentian violet. They are especially abundant in the chlorotic areas of mosaic tissues. They are easily destroyed by certain chemicals, while the motile crystal-like bodies are extremely resistant to 80 per cent alcohol, 30 per cent acetone, and weak acids; but they are sensitive to weak alkalies. Whether these two types of bodies are causal or incidental has not been determined. Certainly, however, they are constantly associated with the destruction of the chloroplasts.

A mycetozoan found in tobacco plants with mosaic-like symptoms. PHILIP M. JONES.

An intracellular mycetozoan has been found in tobacco plants. It occurs only in plants with mosaic-like symptoms. Invaded cells become necrotic and the adjoining ones hyperplastic. Plasmodia may be found in all tissues except bast fibers and xylem.

The plasmodia have been cultured in sterilized Knop's solution from naturally and experimentally infected plants. These plasmodia give rise to amoebae or flagellates with one flagellum. Mitotic division occurs in both. Flagellates are formed also by conjugation in pairs of gametes produced from chromidia discharged by the nuclei of mature amoebae. Both amoebae and flagellates encyst. On germination, the cysts produce amoebae which fuse into plasmodia. These soon become granular and vacuolated and form free spores in a row the nuclei of which discharge chromidia preceding development of three- to sixteen-cyst spores. The cyst spores germinate and produce the amoeba limax. The organism is *Plasmodiophora tabacum* n.s. Similar organisms occur in potato plants affected with either mosaic or leaf-roll and tomato plants affected with mosaic. Work is in progress on the etiological phase of the problem.

Incubation period of aster yellows in its insect host. L. O. KUNKEL.

Transmission experiments indicate that the virus of aster yellows passes through an incubation period in its insect host, *Cicadula sexnotata* Fall. The length of the incubation period has varied somewhat in different experiments. In the case of nymphs a period of two weeks or more must usually elapse before non-viruliferous insects exposed to a diseased plant are able to transmit yellows to healthy plants. The incubation period is somewhat shorter in adult insects where it usually lasts from six to ten days. Viruliferous insects apparently never lose their ability to transmit yellows.

Inhibition of mosaic infection. O. H. ELMER.

Experiments indicate that diluting the juice from mosaic tobacco with juice from healthy plants causes an inhibitory effect on the mosaic virus. Dilutions were made by mixing one cc. filtered juice from mosaic tobacco with nine cc. filtered juice from bean, cucumber, and tobacco plants. Control inoculations were made with the virus diluted in water.

After the mixed juices had stood 7 to 14 days, inoculations of tobacco resulted as follows: Three series of inoculations with virus diluted with bean juice resulted in 6 per cent infection, compared with 100 per cent in the controls. When the virus was diluted with cucumber juice, infection was reduced 53 per cent and the average incubation period was lengthened three and one-half days. Inoculations with virus diluted with healthy tobacco juice resulted in reducing infection 60 per cent and in lengthening the incubation period six days.

When the mixed juices stood 21 days, the percentage infection and incubation period were similar to that in the controls. Such inoculations were made with mixtures of

virus in bean, cucumber, and tobacco juice that had shown marked inhibition ten days previously. It appears from these trials that healthy plant juices produce an inhibitory effect on mosaic virus, but that this effect is not permanent.

The relation of the seed-corn maggot to potato blackleg. J. G. LEACH.

The seed-corn maggot (*Phorbia fusciceps* Zett.) is an agent of dissemination, inoculation, and hibernation of bacteria capable of causing potato blackleg. Bacteria are constantly associated with the insect and are necessary for the normal development of the larvae. Bacteria do not occur inside of the eggs, but are commonly present on the surface. Virulent pathogenic bacteria are present in the puparia and emerge with the adult. Pathogenic bacteria also are commonly present in the intestinal tract and excrement of adult flies. Eggs are deposited in the soil on or near healthy seed-pieces or sprouts. On hatching, the maggots burrow into the seed-piece and inoculate it with bacteria which spread into the stem, causing blackleg. The insect has been responsible for outbreaks of blackleg in fields planted with carefully treated seed from fields known to have been free from blackleg. Control measures have not been devised.

Alternaria tuber rot of potatoes. L. O. GRATZ and REINER BONDE.

Potato tubers were inoculated by stirring them about in foliage spotted with "early blight" (*Alternaria solani*). Infection resulted when the tubers were inoculated and stored under either moist or dry conditions, the latter producing the most typical lesions. High percentages of infection were obtained under all conditions, but the best results were obtained by inoculating moist tubers and drying them before storing. Storage in paraffined cloth bags yielded less tuber infection, fewer lesions per tuber, and smaller lesions than storage in cloth bags. Tubers packed in soil and leaves had fewer spots than those stored in bags. Basement storage yielded higher percentages of infection, more lesions per tuber, and larger lesions than attic storage. Almost no infection was obtained at 25°, and the rate of development was very slow at from 5 to 7° C. The optimum temperature was approximately 15° C. Infection resulted on half grown tubers and throughout the season until after digging time, but was practically nil on tubers inoculated 20 days after digging. Bruising was an aid, but not essential, to infection. The most typical lesions developed when the tubers were not bruised. No lesions were observed on freshly dug tubers. The Spaulding Rose No. 4 was the most susceptible variety, but lesions were observed and produced on other varieties.

Some points of view on the plant virus problem. (Invitation paper) JAMES JOHNSON.

Virus diseases of potatoes. (Invitation paper) E. S. SCHULTZ.

Further studies of potato virus diseases in Idaho. (Invitation paper) C. W. HUNGERFORD and J. M. RAEDER.

Transmission of potato spindle-tuber disease by cutting-knives and seed piece contact. R. W. GOSS.

Successful inoculations for spindle-tuber transmission were made in the greenhouse in 1924 by rubbing together freshly cut surfaces of infected and healthy potatoes. In 1925, under field conditions, 40 inoculations were made by cutting healthy seed with a knife previously used to cut infected tubers, and a similar number were made by rubbing together the cut surfaces of healthy and infected seed pieces. Both the healthy checks and the inoculated seed pieces were from the same healthy tubers and were planted in adjoining rows.

The contact inoculations resulted in 47.5 per cent infection, 45 per cent questionable infection and 7.5 per cent healthy as compared with 32.5 per cent, 47.5 per cent and 20 per cent respectively for the knife inoculations. In addition to the distinct symptoms on the tops and tubers, the yield also was reduced, the average of both sets of inoculations being 153 gm. per plant for the disease checks, 602 gm. for inoculated and infected plants, 962 gm. for those considered questionable infection, and 1154 gm. for healthy checks. Cutting knives and seed piece contact can transmit spindle-tuber, and they may prove to be sources of infection in the field.

Resumé of results of recent investigations on potato diseases. (Invitation paper) FREEMAN WEISS.

Nuclear phenomena and life history of Urocystis cepulae. ALPHEUS WESLEY BLIZZARD.

The chlamydospores of *Urocystis cepulae* germinate, immediately after maturity, by the protrusion of a spherical, hyaline vesicle. This vesicle, the promycelium, buds out a varying number of hyphal branches which develop immediately into a mycelium. Conidia are not produced.

The mycelial cells (the homologue of the conidia) of the saprophytic mycelium may germinate and produce mycelia, thus functioning as oidial spores which serve to propagate the fungus in the soil. The mycelium grown *in vitro* is able to infect onion seedlings. The cells of the saprophytic mycelium are uninucleate. Conjugation of these mycelial cells does not occur.

The parasitic mycelium is intercellular. Certain of the vegetative hyphae are uninuclear, but the hyphal cells of the young sorus are all binucleate. Haustoria do not develop.

The spore-balls originate from sporogenous hyphae, each cell of which is binucleate. The definitive fertile spore takes its origin from a binucleate, centrally located cell of a sporogenous branch, and soon differentiates by a notable increase in size. The young spore dominates the surrounding cells of the sporogenous hyphae which are the primordia of the pseudo (nurse-cells) spores of the spore ball. The two nuclei of the young spore fuse. Thus the mature spore becomes uninucleate.

Infection and spore germination studies with Puccinia sorghi. M. A. SMITH.

Oxalis corniculata was found heavily infected with *Puccinia sorghi* throughout Iowa during 1925. The earliest infection observed was on April 28 and the latest on June 19. Normal aecidial infection was obtained with teleutospores of *Puccinia sorghi* on *Oxalis corniculata, O. europea* and *O. tropaeoloides,* while only pycnidial infection occurred on *O. valdiviensis.* No infection occurred on *O. cernua* or *O. rubra.*

In five trials the uredospores of *Puccinia sorghi* were placed on a dry substratum at a temperature of 25° C., and relative humidities of 90, 95, 96, 97, 97.5, 98.2, 98.7, 99.1, and 100 per cent were maintained. Of the uredospores, 54 per cent germinated at 100 per cent relative humidity; 46 per cent at 99.5; 36 per cent at 99.1; 25 per cent at 98.7; 11 per cent at 98.2, and 3 per cent at 97.5. Beginning September 15, 1924, germination tests were made of the uredospores held in the open. During September and October, 90 to 100 per cent of the spores germinated; 65 per cent in November; 12.5 per cent in December; 4.2 per cent in January; 3 to 4 per cent in February; and only a trace in March. After April 15, none of the uredospores germinated.

Changes in hydrogen-ion concentration induced by carbon dioxide in relation to the germination of spores of Ustilago levis. MARY F. HOWE.

In attempting to analyze the effect of fragments of living plant tissue on the increased germination of the spores of *Ustilago levis,* the influence of the carbon dioxide given off by the tissue on the hydrogen-ion concentration was studied.

Spore suspensions were made by placing the spores in three c.c. of tap water in Syracuse dishes. In such cultures it was ascertained that the highest percentage of germination occurred at pH 4.9. The colorimetric and electrometric methods were used in making this determination. A continuous flow of carbon dioxide liberated into the spore suspension from a Kipp generation caused the pH to vary from 6.7 to 4.9. A similar variation resulted where the source of carbon dioxide was fragments of living plant tissue.

Spore suspensions confined in an atmosphere consisting of different percentages of carbon dioxide failed to produce this optimum of pH 4.9. However, by a continuous flow of carbon dioxide into the spore suspension, this optimum (pH 4.9) was obtained as well as a high percentage of germination comparable to that noted in cultures where fragments of plant tissues were present.

Facultative parasitism of Alternaria and some other fungi. P. A. YOUNG.

Helminthosporium gramineum and many species of *Alternaria* and *Macrosporium* are facultative parasites having wide experimental host ranges. Their failure to infect many plants in nature shows that their host ranges are limited. They usually infected aseptic seedlings in tubes and leaves in the greenhouse within 2–7 days after inoculation or not at all. Mycelium generally projected from the lower sides of *Impatiens pallida* leaf spots. Red infection rings appeared below rings of *Alternaria* spores on sorghum leaves. *Diplodia zeae* caused a rot of wheat plumules. None of the dematiaceous fungi used caused rots of wounded apples. Cabbage leaves covered three days by shaded bell jars underwent great physiological changes, particularly in transpiration control. Susceptibility may be correlated with this change, for dematiaceous fungi infected many plants covered with bell jars, but few in open beds. Bell jars probably did more to augment infection than merely preventing evaporation of water placed on the leaves. *Helminthosporium gramineum* and three species of *Alternaria* acted like rust fungi in causing green spots in yellow leaves of wheat, rye, radish, and nightshade. Perhaps fungous enzymes stimulate formation or delay decomposition of chlorophyll in host cells near such leaf spots.

Infection phenomena of Alternaria, Diplodia, and some other fungi. P. A. YOUNG.

Diplodia zeae, Cephalosporium acremonium, Colletotrichum nigrum, and many species of *Alternaria, Macrosporium,* and *Helminthosporium* induce infected wheat coleoptiles to form spherical or cylindrical, hyaline to brown bodies on and in the cell walls. The name "callosities" has been proposed for these bodies. Their formation is a typical reaction of plumules of wheat, oats, rye, barley, pop corn, sorghum, broom corn, cabbage, radish, turnip, tomato, soybean, wax bean, pumpkin, muskmelon, and *Abutilon theophrasti* to infection by *Alternaria* and *Macrosporium.* No callosities appeared in infected cells of the membranes between onion bulb scales. Callosities partly or entirely enclose narrow penetration hyphae which often connect them to appressoria or internal hyphae. Wheat coleoptiles infected with *Alternaria* characteristically exhibit callosities, isolated or numerous brown cells, swollen cell walls, penetration hyphae, internal hyphae, and, when stained with Congo red, red discs of altered cell-wall material surrounding infection points. Such red discs were seen in wheat cells infected with *Colletotrichum* and *Cephalosporium. Alternaria* caused auto-stained brown discs to appear at infection points in sorghum coleoptiles. Mechanical injury alone caused brown discolorations of wheat, sorghum, and broom corn coleoptile cells. Internal hyphae of *Alternaria* occurred in swollen cell walls of wheat, cabbage, and muskmelon.

Downy mildew (Sclerospora graminicola (Sacc.) Schroet.) on the Everglade Millet (Chaetochloa magna (Griseb) Scribn.). W. H. WESTON, Jr., and G. F. WEBER.

The causal fungus of downy mildew of Everglade millet appears to be *Sclerospora graminicola* (Sacc.) Schroet. It was collected on *Chaetochloa magna* (Griseb) Scribn., growing wild in the Everglades of southeastern Florida. No other host has been found.

Its conidial stage, in size, structure and method of development of the conidiophores, in the structure, germination and quantitative measurements of the zoosporangia (conidia) and the oogonial stage in its development, structure and biometric characteristics, agree in their essential features with the representative material of the species as it occurs typically on *Chaetochloa viridis* in Minnesota and other northern states. This is the first time, however, that this species of *Sclerospora* has been found in the southern states and the first time also that it has been reported on this host in any locality.

The attack of the fungus usually results in systemic infection, but occasionally the disease has been found localized in limited spots on the leaves. During the later stages great numbers of oogonia develop in the mesophyll tissue of the mature leaves which disintegrate and become shredded out into fibrous tangles. Large numbers of oogonia also develop in the inflorescences which grow abnormally into distorted and deformed structures. (Cooperative investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Florida Agricultural Experiment Station.)

Studies on Helminthosporium sacchari on sugar cane in Porto Rico. MEL. T. COOK.

This fungus was first described from Java by Van Breda de Haan in 1892 as *Cercospora sacchari*. Butler transferred it to the genus *Helminthosporium*. The species described from many of the West Indies is considered the same organism as that reported from Java, India, Hawaii, and other places. It is now considered a very destructive disease in Hawaii and Porto Rico.

The first destructive outbreak in Porto Rico was in 1923, and the second in 1925. The development of the disease is influenced by temperature and moisture. It is exceptionally destructive to young plants, killing great numbers of them in some varieties. Its sudden recognition as a serious disease probably is due to the development of new varieties, some of which are very susceptible.

Colletotrichum trifolii and Gloeosporium caulivorum on clover. JOHN MONTEITH, JR.

Investigation of the two common clover anthracnose diseases has shown their distribution largely dependent on temperature. On potato dextrose agar, the optimum temperature for growth of *Colletotrichum trifolii* is about 28° C., whereas the optimum for *Gloeosporium caulivorum* is about 20° C. The lowest favorable temperature for *C. trifolii* was 12° C., while *G. caulivorum* grew at temperatures as low as 4° C. At 32° C. the *Colletotrichum* grew rapidly but the *Gloeosporium* made almost no growth. *C. trifolii* produced spores abundantly at 20° to 32° but not at the lower temperatures, whereas *G. caulivorum* over its whole temperature range gave the typical colonies consisting almost entirely of spores produced by a process of budding, with practically no development of mycelium. Artificial inoculations under controlled conditions indicate that this temperature relationship is also similar for infection. The two fungi produce virtually the same disease symptoms on clover. *C. trifolii* causes most damage during warm weather, and is especially common in the southern states. *G. caulivorum* is usually found in northern sections or in the South during the cooler seasons. Certain imported strains of red clover are most susceptible, while certain American strains are most

resistant to both organisms. (Cooperative investigations between the Office of Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Wisconsin.)

Study of Peronospora trifoliorum DeBy. on species of Leguminosae. M. K. PATEL.

Because of increased acreage of alfalfa, *Peronospora trifoliorum* is assuming greater economic importance in the middle west. Its demonstrated host range is limited to *Medicago sativa* L. and *M. lupulina* L., although 31 species in 16 genera of legumes were exposed to infection with viable conidia of *Peronospora*. Stock cultures are maintained readily in the greenhouse on seedling alfalfa plants. In such cultures conidia and oospores are formed in abundance.

Infection in alfalfa, although usually local, may become systemic, involving parts of the shoots from a crown. *P. trifoliorum* survives unfavorable growth periods by oospores produced sparingly in the leaves of alfalfa in the late fall. Although many attempts have been made to show that the mycelium may live over in the infected plants, no positive data have been secured. Conidia placed in water and allowed to freeze were viable after 173 hours. The optimum temperature for the germination of conidia was 18° C., minimum 4° C., and maximum 29° C. The spores would not germinate on a dry slide in atmospheric humidity of 100 per cent. A film of water was essential for germination. The presence of formaldehyde, acetaldehyde, benzaldehyde, butyraldehyde, furfuraldehyde, toluol, and ethyl alcohol in the germinating chamber inhibited germination of conidia.

A yellows resistant strain of Copenhagen market cabbage, Iacope. FRANK VAN HALTERN and A. T. ERWIN.

A strain of Copenhagen market cabbage, known as Iacope, has been isolated and, in the field, has proved consistently resistant, during the past three years, to the cabbage yellows disease (*Fusarium conglutinans*). Its resistance is variable, ranging from 75 to 95 per cent, depending on the extent of soil infestation and the temperature range.

Iacope, like its parent, has a medium large, round, solid head, less fixed as to shape, color, and time of maturity. The yield of Iacope is fully equal to, if not greater than, that of Copenhagen market. In 1924, it yielded at the rate of 12 tons and in 1925, ten tons per acre. Seed of Iacope is now available through the Muscatine Cabbage Seed Association and several of the leading seed firms in the middle west. The seed-bed method of selection has permitted the building up of its resistance in successive generations. The progeny of selfed, resistant strains has proved equally or even more resistant than the parent.

A new Fusarium resistant cabbage. J. C. WALKER, JOHN MONTEITH, JR., and F. L. WELLMAN.

For some years attention has been given to the development of Fusarium-resistant strains of mid-season varieties of cabbage. The results reported herein pertain to selections made from the variety, Glory of Enkhuizen. The method heretofore practiced of making head-to-row tests from open-pollinated plants has been modified by greenhouse propagation of seed, wherein plants are selfed or close-pollinated artificially. Progress has thus been hastened. Moreover, a line has been isolated which appears to be very uniform in type and practically homozygous for resistance in the field. The evidence of stability in resistance has been shown in two successive generations on

thoroughly infested soil in southeastern Wisconsin, and has been corroborated in trials in a number of other localities. Further evidence is found in the fact that whenever individuals of this line have been crossed with susceptible individuals the F_1 hybrids show practically 100 per cent resistance in each case. Because of slight deviation in type from the standard variety, and in view of the added character of resistance, the new strain is to be given varietal distinction. Steps have been taken to increase the new variety for commercial use as soon as practicable. (Cooperative investigations between the Office of Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Wisconsin.)

Ammonia injury of fruits and vegetables in storage. G. B. RAMSEY and L. F. BUTLER.

Brownish-black discolorations of fruits and vegetables, normal in every respect except color, frequently have been observed on the Chicago market. Discolored onions have been found most often, but peaches, apples, pears, and bananas also have been noted showing a similar type of injury.

The absence of indications of a causal organism, and the consistent association with stored products, indicated that the injury had resulted from exposure to a uniformly distributed, deleterious substance while held in storage. This type of injury was duplicated, in the laboratory, with ammonia. It was demonstrated that the yellow, brown, and red pigment-bearing tissues become brownish-red to dark brown, and in some cases black, when exposed to ammonia. The extent of injury is determined by the amount of ammonia in the air and the duration of the exposure. The injury may be merely a blemish, as in the discoloration of the outer dry color-bearing scales of the onion; or it may be serious, as in the softening and browning of the peach. Tables showing the degrees of injury of the products mentioned, when exposed to known strengths of ammonia for varied time intervals, are included in the complete manuscript. (Contribution from the Research Laboratory on Market Diseases of Fruits and Vegetables; United States Department of Agriculture, Bureau of Plant Industry, and the Botany Department, University of Chicago, cooperating.)

Chemical injury to watermelons in transit. W. W. GILBERT and F. C. MEIER.

Railroad companies and shippers have frequently reported injury to watermelons caused by contact with chemicals, including fertilizer materials on the floor and walls of freight cars.

Preliminary experiments were conducted last fall to determine whether any chemicals carried as freight can damage melons and if so under what conditions and the character of the lesions. To approximate shipping conditions, both wet and dry chemicals were used. Some melons were placed directly on the chemicals, while others were separated by building-paper or a layer of straw. Pressure equivalent to that normally received in the car was applied to half the melons. After 5 days, melons under pressure showed marked injuries (discoloration, pitting, softening) when in direct contact with the following moistened chemicals: acid phosphate, potassium muriate, salt, and sodium nitrate. Some damage under the same conditions resulted from ammonium sulfate and potassium sulfate. Neither hydrated nor air-slaked lime was injurious. Slight damage occurred when ammonium sulfate and salt were used dry and no pressure was applied.

Melons under pressure, when protected from wet chemicals by layers of building paper, were not so severely affected. Where straw was used for bedding, even less injury resulted.

Further evidence of the non-transmissibility of the so-called sweet potato mosaic. JAMES L. WEIMER.

Nancy Hall sweet potatoes from vines showing symptoms of mosaic were carried through three generations under different environmental conditions, the mosaic symptoms being evident to about the same degree on each. Healthy plants, with vines growing intermingled with those of diseased plants, gave no evidence of disease even in the second generation. Unsuccessful attempts were made to transmit the disease by the usual mechanical means, as well as by grafting halves of diseased and healthy potatoes. Likewise, portions of diseased vines were grafted onto healthy roots and vice versa. The grafts, following a good growth in the greenhouse, were set in the field. The healthy vines, after growing on diseased roots for eight months, were still healthy, and the diseased vines on healthy roots remained diseased. A small crop of potatoes was produced by all of the plants. Whenever these potatoes sprouted before being dug the young plants were diseased if attached to a diseased root, and healthy if from a healthy root.

Sweet potato mosaic and its incubation period of two growing seasons. H. R. ROSEN.

In his first communication on sweet potato mosaic in 1920, the writer called attention to the non-communicability of this disease. Macerating diseased material and applying it in various ways, including rubbing of young and old leaves, hypodermic injections into stems, petioles, and midribs, heavy applications into cavities made in fleshy roots, then used for propagation, and grafting healthy shoots on diseased plants all resulted negatively. This was true irrespective of time of inoculation or age of inoculated plants. Young as well as old plants failed to show any signs of disease during the growing season. This work has now been confirmed by other investigators, and certainly sweet potato mosaic is not communicable as far as concerns the development of recognizable symptoms during one growing season.

Experiments made by the writer for several years show that the disease has a remarkably long incubation period and, even under optimum conditions, no symptoms appear in less than two successive growing seasons. Furthermore, there usually is a gradual increase in the severity of the disease in the progeny of inoculated plants, although sometimes the symptoms are pronounced when the disease first appears.

Fusaria causing bulb-rot of onions. GEO. K. K. LINK and ALICE A. BAILEY.

Several species of *Fusarium* cause bulb-rot of onions. Two hundred isolations have been made from bulbs collected in the field and market. The pathogenicity of half of these cultures has been tested, but the taxonomic position of all has not yet been determined.

The species which invariably are pathogenic belong to section Elegans. The following have been determined: *Fusarium zonatum* Sh. forma I Wr. (*Fusarium cepae* Hanz. emend. Walker and Tims); *Fusarium zonatum* Sh. forma II nova forma; *Fusarium cepae* Hanz. emend. Link and Bailey. One member of section Liseola, *Fusarium moniliforme* Sheld., isolated from decaying bulbs, is not invariably pathogenic (50% +).

Lesions vary from a mealy, vinaceous buff, to a powdery, dry (mummied) avellaneous to cinnamon decay. Not all Fusaria isolated from onions affected with bulb-rot are pathogenic. Some, when associated with bacteria, cause a decay unlike the typical bulb-rot produced by pathogenic bacteria alone, but like that produced by pathogenic Fusaria when bacteria are present. Identified species of section Elegans which behave in this way are: *F. oxysporum* Schlecht., *F. vasinfectum* Atk., and *F.*

bulbigenum Cke. and Mass. *F. martii* Appel and Wr. var. *minus* Sh. of section Martiella behave similarly.

(Cooperative investigations between the Office of Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Botany Department, The University of Chicago.)

Pseudomonas (Phytomonans)pisi Sackett, the cause of a pod spot of garden peas. C. A. LUDWIG.

A pod spot of garden peas, first discovered in 1922, has been found due to an organism believed to be a strain of *Pseudomonas (Phytomonas) pisi* Sackett. The organism may live in culture and retain its infective capacity without being transferred for more than a year, under some circumstances at least. It can attack stems and leaves as well as pods. It seldom infects, however, except at some point of injury. It infects young, succulent tissues more readily than older, drier ones, and some varieties more freely than others. Culturally, it behaves very much like *Pseudomonas pisi.* The discovery or development of resistant varieties is suggested as the most promising approach to its control.

A wilt disease of peas in Wisconsin. MAURICE B. LINFORD.

A wilt disease of canning peas caused losses in Wisconsin during 1924 and 1925 second only to those caused by *Aphanomyces* root-rot. This newly-observed disease is characterized by early and rapid wilting of leaves with or without previous yellowing, and without decay or lesions of the cortex of roots or basal stem. It develops, characteristically, roughly circular patches throughout a given field. These appear early, and may continue to enlarge until the crop is harvested. Wilt is most frequently destructive in fields which have been heavily cropped with peas. Vascular discoloration occurs frequently but not constantly. Fungal invasion of the vessels of the taproot and lower internodes is characteristic.

Isolation of fungi from the vessels of wilted plants from 68 collections of peas from 18 counties in Wisconsin yielded cultures which fall into three groups, regarded tentatively as three undetermined species of *Fusarium.* Seventy-five cultures from one of these groups have proved pathogenic, while no pathogenicity has been demonstrated in the other two groups.

Green Admiral, a canners' variety, appears highly wilt-resistant, and certain newer varieties promise equal or greater resistance.

Aspergillus decay of cotton bolls. MICHAEL SHAPOVALOV.

A peculiar decay of cotton bolls has been prevalent during the last few years in the southwestern states. It appears at first as a soft, pinkish, circular spot and may spread over the entire boll. The latter prematurely dries up and becomes filled, and covered on the outside with black masses of spores which give it the appearance of being affected by smut. It is erroneously known as smut in some cotton-growing sections of California. The fungus causing this decay is *Aspergillus niger.* The infection is sometimes associated with insect wounds, but frequently such injuries are not apparent. Successful inoculations have been made of green-picked bolls in the laboratory, as well as of growing bolls in the field, by inserting spores of the fungus in scalpel stabs or in needle pricks. Up to 100 per cent of infection has been obtained in moist chambers and as much as 76 per cent in the field.

Cotton wilt studies. V. H. YOUNG.

Strains of *Fusarium vasinfectum* from several states showed an appreciable difference in their pathogenicity for cotton, and appeared to be distinct strains. Some proved extremely pathogenic; others much less so. Later trials showed great variations, the last ones resulting in almost no infections, apparently because soil temperatures were too low. Loss of pathogenicity in culture must also be considered as a possibility.

Inoculations with a monosporic strain of *Fusarium vasinfectum*, under ordinary greenhouse conditions during late summer, and in soil temperature tanks at 30° C. gave 50 per cent infection under the latter condition and none under the former. Experiments in the temperature tanks, using temperatures from 22½° to 35° C., gave no infection at 25°, some infections at 27½°, and the greatest number at 32½° C. There was still infection at 35° C. A second experiment gave similar results. The maximum soil temperature for cotton wilt infection is higher than 35° C. and the minimum temperature is near 25° C. Results are not definite as to the optimum point for infection.

Control of brown-patch in turf. JOHN MONTEITH, JR.

There are two types of brown-patch occurring on lawns and putting greens. Large brown-patch is generally limited to periods of hot, humid weather but the small-spot type may occur over a much wider range of climatic conditions. The effect of fungicidal treatments is different with these two diseases. Large brown-patch may be readily controlled by spraying or dusting with Bordeaux or other copper sprays. Repeated applications of copper salts, however, produce an accumulation of toxin in the soil which may seriously injure turf. Small brown-patch is checked by neither organic nor inorganic copper sprays. All the mercury compounds tested, Uspulun, Semesan, Germisan, Corona 620, Corona 640, and mercuric chloride, controlled both types of brown-patch. The period of protection given by the mercury salts varies from a few days to several weeks, depending on the amount applied, severity of disease attacks, soil, rainfall, and other environmental factors. Formaldehyde, under certain conditions, may protect grass from these diseases for a few days, but its effect is not as lasting as that of the mercury applications. Sulphur does not check either type, and is toxic to grass.

Studies on leafhopper injury to apple leaves. A. A. GRANOVSKY.

During the past three years the leafhoppers of cosmopolitan food habits such as *Empoasca mali* (Le Baron), *E. flavescens* (Fabricius) and *Cicadula sexnotata* (Fallen) were present in abundance in apple orchards of the Peninsular region of Wisconsin, the first being the most abundant. At the same time various degrees of apple leaf injury have been observed which could not be ascribed to any well known cause. It manifested itself in characteristic marginal discoloration of the leaves which gradually progressed downward along the lateral veins, often reaching the midrib. This discoloration is invariably more rapid on the basal than the apical side of the veins. In case of severe injury, the tips and the distal margins of the leaves curled upward, much resembling the "hopperburn" injury of potatoes.

Experiments with caging leafhoppers over the terminal spurs of apples indicate that this type of discoloration is due to the feeding of leafhoppers. Prolonged confinement intensifies the symptoms. Needle inoculations with the macerated leafhoppers produced similar symptoms. The sap of diseased leaves gives a relatively low percentage of infection. The sap of uninfected leaves does not produce the characteristic symptoms. It is believed that this type of apple leaf trouble is due to feeding of leafhoppers and probably to some toxic or infectious agent.

Migration and transformation of Bacillus amylovorus in apple tissue. E. L. NIXON.

The migration of *B. amylovorus* was traced from needlepoint infection to canker formation. Migration of the organism in the form of zoogloea was found in the early stages taking place through inter-cellular spaces in the cortical regions. Subsequently, the organism penetrates the walls of the phloem parenchyma and medullary-ray cells, occupying the space between the walls and the protoplast. In some cases, it further penetrates the protoplast of healthy cells and forms a cyst within the central vacuole. In this condition the organism hibernates. With renewed growth of the host in the spring, the cysts imbibe moisture and disintegrate. The bacterial masses then escape from the host cells. These masses uniting with others then appear as the familiar exudate. In tracing the migration, it was observed that in certain regions the bacterial masses appear to disintegrate. In other regions involution and spore forms occurred.

Studies of apple scab infection under controlled conditions. G. W. KEITT.

An inoculation chamber has been devised in which a saturated atmosphere can be maintained at a wide range of constant temperatures. Leaves of potted apple plants were inoculated by means of ascospores of *Venturia inaequalis* (Cke.) Wint. naturally discharged from apple leaves suspended in the upper part of this chamber. Experiments thus far performed show that the minimal temperature for infection is not more than 6° C. and the maximal not less than 26°. It is probable that further work will extend the lower temperature limit. The optimal temperature for infection was near 20° C., which agrees closely with the optimum for germination of ascospores and growth of *V. inaequalis*. When inoculated plants were held at constant temperatures, the minimal periods of incubation observed at the different temperatures varied from 17 days at 8° C. (lowest temperature tried) to 8 days at 20°.

The minimal numbers of hours of continuous wetting which have thus far yielded leaf infection by ascospores at the temperatures stated are as follows. 6° C., 18; 9°, 13; 15°, 8.5; 20°, 6; 26° 10.

Studies are in progress on the relations of temperature and moisture to the effectiveness of fungicides commonly used to combat scab.

Studies on the development of the ascigerous stage of Venturia inaequalis in nature. G. W. KEITT and E. E. WILSON.

Conditions affecting the abundance of ascospores of *Venturia inaequalis* and the time of their maturity and discharge are of much potential importance in relation to the development and prevention of epidemics of apple scab. Consequently, the development of the fungus at Madison, Wis., was studied from a time prior to leaf-fall until ascospores were matured and discharged. A marked relationship was observed between the time of leaf-fall and ascospore maturity. Under the conditions studied, ascospores matured much earlier in leaves which fell in early autumn than in those which remained on the tree until late autumn or early winter. Observations and experiments are in progress relative to the possible bearing of the time of leaf-fall upon control measures.

Temperature and moisture were shown to be factors of cardinal importance in determining the rate of development of ascocarps.

Infected leaves were sprayed with various chemicals shortly before leaf-fall in the hope of finding a treatment which would prevent or limit the development of the ascigerous stage of the scab fungus without serious injury to the host plant. The results thus far obtained offer sufficient promise to justify continuation of the experiment.

Studies of crowngall and callus overgrowths on apple grafts. A. J. RIKER and G. W. KEITT.

Isolation studies were made on 227 apple trees, representative of lots of stock submitted as crowngall rejects by 12 nurseries in six states. Less than two per cent of these trees yielded *Bacterium tumefaciens* Smith and Town. Tests of the isolation technique on known crowngall material gave positive results from 44 to 46 plants.

A study of the trees which failed to yield the crowngall organism showed most of the malformations to be associated with poorly fitted grafts. Various types of misfitted grafts were grown experimentally in comparison with well-fitted grafts. Malformations could be induced or prevented almost at will by the type of fit. An examination of samples of freshly prepared commercial grafts from eight nurseries showed misfits in sufficient numbers and types to account for all the rootknot reported by these nurseries. The following types of misfit appear to lead commonly to the more severe callus overgrowths: cion tip long; cion larger than stock; graft too loosely wrapped.

Wrapping grafts with cloth, waxed paper, or medical adhesive tape reduced the amount of callus development.

The results thus far available give promise that the major portion of the callus overgrowths at the unions of grafted apples may be prevented by modifications in grafting practice.

Aerial galls of quince. J. A. MCCLINTOCK.

In connection with crowngall studies in Tennessee, two old quince trees, showing numerous well developed aerial galls, have been under observation since the fall of 1924. Attempts to obtain the crowngall bacterium from these galls have been unsuccessful. Hard-wood cuttings made from galled branches of various ages were placed in a sand bench in the greenhouse for comparison with similar quince cuttings from younger, gall-free trees. Repeated examination of the cuttings disclosed no additional gall development, but in all cases where roots did develop on the galled cuttings they grew out from the galls. This indicates that the aerial galls on these old quinces are not crowngalls, but that they are similar to the "burr-knots" of apple and quince described by Swingle.

Hairy root of apple seedlings. J. H. MUNCIE.

Three distinct types of hairy root have been observed on French apple seedlings, namely: (1) the woolly knot form, arising from a distinct gall; (2) the simple form described by Stewart; (3) the type in which fine fibrous roots arise in clusters from the tap root.

The first type is infectious, and has been induced by artificial inoculation of healthy seedlings in steamed soil with *Bacterium tumefaciens*, and by growing healthy seedlings in soil artificially and naturally infested with that organism. The second type was the least common, occurring on seedlings grown from treated seed in steamed soil. The third form was the most common, occurring on seedlings in steamed soil as generally as in check plots receiving no treatment. The percentage of this type was not increased by infesting steamed soil with *Bacterium tumefaciens*. Repeated attempts to isolate *Bacterium tumefaciens* from type three have been unsuccessful, and histological studies have failed to show the presence of an organism within the hairy roots or near the point from which they arise. These last two types on seedlings seem to be non-pathogenic forms of hairy root.

The effect of crowngall on young apple and peach trees and longevity of Bacterium tumefaciens in the soil. J. H. MUNCIE.

Work has been continued on water conduction in galled and normal piece-root grafted two-year "cut-back" Wealthy, Jonathan, and Salome apple, and budded two-year old Carman, Salwey, J. H. Hale, and Elberta peach trees. In 206 isolations from 84 galled apple trees used in these studies, *Bacterium tumefaciens* was recovered in four cases. In 200 apple trees examined by means of longitudinal sections through the middle of the union, 81 per cent of the healthy showed a perfect union of stock and scion, while 63 per cent of the galled showed only partial continuity.

The reduction in water flow through galled Wealthy, Salome and Jonathan trees was 69.7, 21.7, and 47.2 per cent respectively. Lateral roots arising from above or opposite the gall may in some cases counteract the obstruction offered by the gall. In peach the average reduction in water flow through the galled trees is 82.4 per cent.

Young tomato plants were set in soil infested with *Bacterium tumefaciens*, and inoculated by injuring the stem at the surface of the soil and smearing the wound with the infested soil. Typical crowngall resulted up to 102 days after infesting the soil.

A wound dressing for plants. I. E. MELHUS.

A wound dressing possessing many desirable characteristics has been made from the resinous products resulting from refluxing furfural and creosol. It is made by mixing one pound each of commercial creosol and furfural (concentrated) with 0.32 ounces sodium bicarbonate in a reflux condenser and boiling until a resin forms. When it becomes slightly brittle on cooling in a thin film on a glass rod, discontinue boiling and pour the sirupy mass into a shallow pan to cool quickly. Dissolve this resin in equal parts of acetone and furfural. Dissolve four grams mercuric cyanide in a small quantity of acetone, and add it to the resin-acetone-furfural mixture.

This dilute resinous mixture, about the consistency of shellac, can be applied to a wound with an ordinary paint brush. It dries quickly, forming a smooth, dark brown, amorphous coating both on living and dead tissue. The rate of drying and penetration is determined largely by the proportion of acetone to furfural. The plastic and amorphous properties can be controlled by varying the degree of condensation of the resinous product. Dead tissues are penetrated from one to two inches by the furfural in the dressing, while living tissues are not penetrated nearly as extensively by the furfural. The resin remains largely on the surface of the wound.

Control of bacterial spot of peach with sodium silicofluoride. H. W. ANDERSON.

Spraying experiments on peach for control of bacterial spot (shot hole) during 1925 at Urbana, Illinois, demonstrated the possibility of control with a spray of sodium silicofluoride in water. Preliminary laboratory tests proved this substance effective in preventing growth in broth cultures in dilutions of 1–3,000. Seven applications were made, ten to fourteen days apart. Various concentrations were used. One containing two pounds in 50 gallons of water proved satisfactory. The checks showed from 10 to 90 per cent diseased leaves on October 1, while very few infections could be found on the sprayed trees. It is probable from the dates of infection that only three of the seven sprays would have been necessary for control. Sulfur sprays first gave increased infection over the checks, due probably to wetting the trees during spraying. Later, the checks were more heavily infected. No injury to the foliage resulted from the use of the sodium silicofluoride, neither in the orchards nor in the greenhouse where preliminary tests were made. This season's weather conditions were so abnormal, how-

ever, that no assurance can be given that injury will not result in other seasons. Copper sprays, including two brands of "colloidal" copper and Bordeaux mixture, caused serious spray injury.

Observations on masking of raspberry mosaic by high temperature. R. B. WILCOX.

Certain types of raspberry mosaic are masked during hot weather. Mottled leaves formed during cool weather retain their mottling, but new leaves developed during hot weather appear normal. Temperature-control chambers were not available to the author for a study of this masking.

Rubus innominatus, infected with one type of transmissible mosaic, was found to be not only peculiarly sensitive to temperature changes, but recorded these changes by very conspicuous leaf markings. Such infected canes, accompanied by a record of their growth during the summer, showed correlation between the appearance of seemingly healthy leaves and the occurrence of periods of high maximum temperatures. Study of this relationship indicates that for this mosaic of *Rubus innominatus* the critical temperature is approximately 75° F. (24° C.).

A citrus bacteriosis occurring in South Australia. H. K. LEWCOCK.

Intermittently during the last decade, particularly in wet seasons, a bacterial disease of citrus fruits has manifested itself in the fruit-growing districts of South Australia and Victoria. In some instances, losses amounting to 30 per cent to 40 per cent of the crop have been recorded. Such heavy infection, however, has been restricted to districts with moderately heavy winter rainfall, *viz.*, 20 to 30 inches.

The disease develops on tree-ripe fruit during the winter and spring months. In the early stages, the dry, reddish-brown lesions are circular or oval in outline, but may rapidly extend over a large part of the surface of the fruit unless dry weather intervenes. The diseased tissue is depressed somewhat below the bottom of the normal oil glands into the white portion of the rind, but does not extend into the pulp. The line between the healthy and diseased tissue is distinct and sharply defined.

The disease is caused by *Pseudomonas citriputeale,* first described by C. O. Smith from Riverside, California, in 1913, under the name of "Black Pit of the Lemon." In 1924 the author recorded it from Australia.

Preliminary notes on avocado fruit decay. WM. T. HORNE.

Rhizopus nigricans Ehr. causes a wound decay of ripe fruit of avocado, and the mycelium is capable of slowly penetrating the uninjured surface. The seed is not injured. A fungus, probably *Diplodia natalensis* Ev., from Florida fruit, causes active dark decay and sometimes destruction of the seed with dry rot. *Colletotrichum gloeosporioides* Penz. and *Pestalozzia* Sp. are conspicuous in decaying Florida fruits. California fruits decay mainly from wound infection with species of *Alternaria, Cladosporium, Fusarium,* and similar molds. The rots produced are slow-acting and rather dry. Bacteria of various types are abundant in late stages of decomposition, but apparently are not pioneers in tissue invasion.

The take-all disease in Canada. W. P. FRASER, P. M. SIMMONDS and R. C. RUSSELL.

In an extensive field survey made during 1923–25 the "take-all" disease of wheat was found causing considerable loss in a large number of spring wheat fields throughout the semi-wooded part of northeastern Saskatchewan, between latitudes 52 and 53 and as far north (25 miles) as was surveyed. "Take-all" was found also in north-

central Alberta. The disease was not observed in the prairie areas. "Take-all" occurred on the first crop after breaking, and was more severe on the second crop and in fields devoted to wheat a number of years. Mature perithecia of what is apparently *Ophiobolus cariceti* have been found on *Torresia odorata*. The mycelium of the same fungus was found on *Bromus* spp., growing in wheat fields. From observational evidence, it is believed that the "take-all" fungus is indigenous to this area, and parasitizes a number of the native grasses, particularly those with rhizomes. In the greenhouse, the Saskatchewan fungus attacked all of the members of the subspecies of *Triticum* tested. In addition to the susceptible grasses noted by Kirby, the following were attacked: *Avena Hookeri, Bromus inermis, B. latiglumis, B. Porteri, Agropyron dasystachyum* and *Elymus innovatus*.

The influence of carbon dioxide and oxygen on the growth of Ophiobolus graminis in pure culture. MURLEY FELLOWS.

By varying the CO_2 and oxygen content of the atmosphere it was found that *Ophiobolus graminis* grows in oxygen contents varying from 0.2 to 21.3 per cent in both the liquid and solid media. In the decoction, a gradual diminution occurred as the oxygen percentage was lowered. On agar, no marked diminution obtained until the oxygen was below 6 per cent.

A variation in the carbon-dioxide content between 0.9 and 18 per cent did not affect the growth of *Ophiobolus graminis* greatly. Eighteen per cent reduced the growth somewhat. In all the carbon-dioxide experiments, the growth curve was bimodal. The oxygen content of the various carbon-dioxide concentrations was kept near normal.

It is not believed that the variations in carbon dioxide and oxygen as found in arable soils are great enough to materially affect the growth of *Ophiobolus graminis*. (Investigations conducted by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, with the Wisconsin and Kansas Agricultural Experiment Stations cooperating.)

The effect of tillage, fertilizers, and rotations on the spread of wheat foot-rot. LEO E. MELCHERS and M. C. SEWELL.

Two years' additional data on winter wheat tillage plots confirm the conclusion reached in a recent publication, namely, that shallow and late tillage checks the rapid spread of foot-rot, while deep, early plowing favors its survival and spread. Furthermore, plots, disced during the summer to keep the weeds down, showed only light infection; keeping the weeds scraped off without further stirring the soil, or merely discing at seeding showed no appearance of foot-rot. There appears to be an equal spread where, (1) stubble is burned before plowing, (2) where straw is applied and plowed under, (3) where straw is applied and disced under. Where straw was applied in winter as a top dressing, foot-rot spread uniformly over the entire area treated.

A series of fertilizer plots, devoted to wheat since 1919, with cowpeas as a green manure catch crop every third year, developed foot-rot in 1922. These plots receive various applications of phosphate, potash, nitrogen, and barnyard manure, and are usually plowed early and deep. The application of these various fertilizers seems to have had little influence on the spread of foot-rot during four years' observations. (Cooperative investigations by the Kansas Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

The influence of temperature upon the metabolism and expression of disease resistance in selfed lines of corn. JAMES G. DICKSON and JAMES R. HOLBERT.

Disease resistant and susceptible strains of Yellow Dent corn have been selected from selfed lines of corn and self-pollinated until practically homozygous. These strains have been studied comparatively under controlled environmental conditions as well as under varying field conditions. The resistant strains remained healthy over a much wider range of temperature than open-pollinated material or the susceptible strains as shown in the following table.

Strains of Corn	Temperature range of resistance to seedling blight in degrees C.
Resistant selfed lines	16–32
Selected open-pollinated	24–32
Susceptible selfed lines	32

The metabolism of the resistant, selfed strains, although similar to that occurring in the open-pollinated corn, is stable over a greater range of temperature. These resistant strains are high in hexose substances and low in pentosan materials at 16° C. and higher. In contrast, the open-pollinated corn maintains this high ratio of hexose substances at temperatures of 24° C. and higher.

The metabolism of the susceptible, selfed strains does not correspond to that of either the resistant strains or open-pollinated corn. The susceptible strains studied so far are different in composition and in relative ratio of substances present at all temperatures. In general, the pentosan substances increase with the rise in temperature rather than decrease as in the resistant strains.

(Cooperative investigations by the Wisconsin Agricultural Experiment Station, the Funk Brothers Seed Company, and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

Seed treatments for the control of certain diseases of dent corn. JAMES R. HOLBERT, CHAS. S. REDDY and BENJAMIN KOEHLER.

Extensive experiments conducted at various places in Illinois during the past five years have shown that dent corn grown from seed infected with *Diplodia zeae* and *Gibberella saubinetii* is greatly improved in both yield and quality by seed treatment, while corn from *Fusarium moniliforme* infected and scutellum-rotted seed has not been improved. Only under certain soil conditions, not fully understood, have seed treatments increased the yield of corn grown from seed infected with *Cephalosporium acremonium*. Treated, infected seed has not yielded as well as untreated, nearly disease-free seed. Seed treatment may prove important when it is impossible to secure sufficient nearly disease-free seed. The summarized results follow.

Seed treatment materials	Total No. replications	Nearly disease-free seed		Diplodia-infected seed		Gibberella-infected seed	
		Incr.	Decr.	Incr.	Decr.	Incr.	Decr.
		Bu.	Bu.	Bu.	Bu.	Bu.	Bu.
Uspulun (0.5%)	317	.....	0.5[a]	15.6	.....	15.1	.....
Bayer Comp. "	24	.....	2.1[a]	19.3	.....	7.0	.....
Semesan "	72	.....	0.5[a]	6.4	.....	18.2	.....
DuPont No. 12 "	72	.....	3.8	14.0	.....	.....	.....
Mercury A "	36	.....	1.8[a]	16.4	.....	19.8	.....
Corona 620 (0.15%)	120	.....	3.1[a]	21.7	.....	17.6	.....
Germisan "	80	.....	1.7[a]	17.4	.....	.....	.....
Standard Oil H_1 (100%)	42	0.2[a]	.....	13.1	.....	17.9	.....
DuPont No. 12 (Dust)	42	5.1[a]	.....	9.7	.....	12.1	.....
" " 18 (Dust)	24	.....	3.7[a]	10.7	.....	16.6	.....
Semesan (Dust)	12	.....	6.6[a]	9.6	.....	.....	.....
Semesan, Jr. (Dust)	30	.....	1.3[a]	20.7	.....	19.0	.....
Bayer Dust	30	8.5	.....	15.8	.....	28.9	.....
Corona 640 S (Dust)	27	.....	3.1[a]	8.5	.....	19.8	.....
S. F. A. No. 225 (Dust)	12	8.2	.....	16.8	.....	.....	.....

[a] Odds less than 30: 1, others greater.

(Cooperative investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, the Funk Brothers' Seed Company, and the Illinois Agricultural Experiment Station.)

The effect of the alternate hosts on physiologic forms. S. M. DIETZ.

The effect of *Rhamnus* species on the fixity of physiologic forms of *Puccinia coronata* was studied to determine the possible influence of these alternate hosts on the infective capabilities of the aecidiospores produced on them and the possible increase of their gramineous host range.

It previously has been shown that *Puccinia coronata avenae, P. coronata calamagrostis, P. coronata festucae,* and *P. coronata holci* will not produce normal uredinial infection *inter se.* However, aecidiospores from *Rhamnus caroliniana, R. cathartica, R. lanceolata, R. pinetorum, R. smithii* and *R. rubra,* produced by teleutospores from *Avena sativa,* formed normal infection on *Nothoholcus lanatus* under controlled greenhouse conditions. Aecidiospores from *Rhamnus lanceolata,* inoculated with teleutospores from *Calamagrostis canadensis,* produced abundant uredospores on *Avena sativa* in nine trials conducted over three years. Aecidia on *Rhamnus lanceolata* produced by teleutospores on *Avena sativa* formed abundant uredospores on *Calamagrostis canadensis.* Aecidia collected on *Rhamnus alnifolia* in the open produced abundant uredinial infection on *Avena sativa.*

Aecidiospores from *Rhamnus lanceolata,* produced by teleutospores from *Festuca elatior,* formed abundant uredospores on *Festuca interrupta, Hordeum murinum,* and *Phalaris stenoptera* and a lesser degree of infection on *Andropogon trinii, Bromus inermis, Lolium subtratum, Phalaris angusta, P. arundinaceae, P. boehmeri, P. brachystachys, P. bulbosa, P. nodosa, P. truncata,* and *Stipa* sp. The above recorded grasses produced no infection when repeatedly inoculated with uredospores of *Puccinia coronata*

festucae. (Cooperative investigations by the Iowa Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

Alternate hosts of Puccinia coronata II. S. M. DIETZ.

Rhamnus alpina, R. chlorophera, R. ilicifolia, R. infectoria, R. smithii, and *R. tinctoria* are susceptible to *Puccinia coronata festucae.* When inoculated with teleutospores of crown rust from *Festuca elatior, Rhamnus dahurica, R. dahurica* var. *nipponica, R. frangula,* and *R. japonica* became infected, but did not produce aecidia. *Rhamnus japonica* showed no infection with the above physiologic form. *Berchemia scandens* and *Zizyphus lycoides* showed pycnidial infection, while *Caenothus americanus, Elaeagnus angustifolia, Lonicera flava,* and *Zizyphus obtusifolia* were copiously flecked by *Puccinia coronata festucae. Lonicera flava* also was markedly flecked by *Puccinia coronata calamagrostis.*

Local epidemics of crown rust were started again during the spring of 1925, in the Upper Mississippi Valley, by *Rhamnus cathartica* and *R. lanceolata.* The latter produced the heaviest aecidial infection during the past nine years in the spring of 1925.

One hundred and fifty-eight additional plantings of *Rhamnus cathartica,* 69 of which were urban and 89 rural, were located during 1925. These plantings contained 11,057 counted bushes besides 5,380 feet of hedge where the bushes were not counted. (Cooperative investigations by the Iowa Agricultural Expriment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

Physiologic specialization of Ustilago zeae and Puccinia sorghi and their relation to corn improvement. E. C. STAKMAN and J. J. CHRISTENSEN.

Ustilago zeae is one of the destructive pathogenes of corn. Apparently the only method of reducing losses appreciably is the production of resistant lines. However, there is circumstantial as well as direct evidence that there are several physiologic forms of the organism. This fact is likely to complicate the problem of developing resistant varieties. *Puccinia sorghi* is not considered a destructive pathogene. However, in 1925, it did considerable damage on certain selfed lines of corn in the plant breeding plots at the University of Minnesota. It is possible, therefore, that corn rust may become an important factor in corn production if susceptible sorts are produced and grown commonly. Some lines of a given variety of corn are extremely resistant, while others are highly susceptible. The rust situation is also complicated by the existence of several physiologic forms. At least three, and possibly five, have been recognized by their action on eight selfed lines of corn. A very virulent form was collected in Oklahoma, Kansas, Nebraska, and New Hampshire, while a less virulent one was obtained from several localities in Minnesota, and a very weak one from Texas. There is no correlation between rust resistance and smut resistance of selfed lines. (Cooperative investigations by the Section of Plant Pathology and the Section of Plant Breeding, Department of Agriculture, University of Minnesota.)

Physiologic specialization of Fusarium lini, Bolley. W. C. BROADFOOT and E. C. STAKMAN.

Fusarium lini consists of several physiologic forms. At least eight are distinguished by their parasitic action on the following varieties of flax: Primost, Minn. 25 (C. I. 177); Winona, Minn. 182 (C. I. 179); N. D. 3080 (C. I. 275); N. D. 40013 (C. I. 241). Six of the forms differ greatly from each other in virulence. Two are about equally virulent, except that one consistently causes loss of chlorophyll above the cotyledons in all varieties of flax inoculated. It also causes loss of chlorophyll in seedlings of barley, oats, and rye.

The cultural characteristics of some forms differ from those of others, but this is not a safe criterion for the determination of all of the forms. Neither can the forms be differentiated by rate of growth at different temperatures. There are differences in size and septation of spores of different forms grown under the same conditions, but there also are similar differences when the individual forms are grown under different conditions. Therefore the forms cannot be differentiated by spore morphology. Some of these forms infect heavily certain resistant varieties of flax, but it is not yet known how important this fact is in controlling flax wilt through the development of resistant strains.

A strain of sorghum kernel smut which infects milo and hegari. W. H. TISDALE, L. E. MELCHERS, and H. J. CLEMMER.

In comprehensive experiments with sorghums completed in 1921, milo, feterita, and hegari were found to be almost immune from kernel smut. In 1923 kernel smut was reported in milo in southwestern Kansas, northeastern New Mexico, and northwestern Texas. Milo and hegari were found to be smutted in this region in 1924. Experiments conducted cooperatively by the offices of Cereal Investigations and Dry-Land Agriculture, Bureau of Plant Industry, United States Department of Agriculture, and the Kansas Agricultural Experiment Station in 1924 and 1925 have shown that the smut from milo and hegari infects pure lines of milo and hegari and the sorghums susceptible to the commonly occurring strain of *Sphacelotheca sorghi.* It does not infect feterita. Covered kernel smut from the susceptible sorghums grown at Manhattan, Kansas, failed to produce smut in milo or hegari as in previous experiments. Infected heads of milo and hegari often are only partially smutted by this new strain of smut. The fact that a kernel smut now exists which attacks hitherto resistant varieties greatly complicates the problem of breeding for resistance. Although there are certain characters of the sori of this smut and the sterile cells of the fungus which resemble *S. cruenta,* it is more typical of *S. sorghi.*

A Pythium seedling blight and root rot of dent corn. HELEN JOHANN, J. R. HOLBERT, and JAMES G. DICKSON.

During the past few years an undetermined species of *Pythium* has been found associated with root rots of dent corn in Illinois and Wisconsin. It is somewhat closely related to the species causing root rot of cane, as described by Carpenter, in that it has lobulate sporangia and smooth oogonia and oospores.

Infection usually occurs at the tips of rootlets and proceeds proximally, producing a soft rot, involving first the cortex and later the vascular elements. Unlike infections by *Diplodia zeae* and *Gibberella saubinetii,* the mesocotyls are not attacked until roots are practically destroyed.

Soil temperatures of 12–16° C., and high soil moistures, tend to favor blighting which prevents germination. Some seedling blight may occur above 16° C. If the infection is not severe enough to kill the seedlings, they may be more or less retarded in size and vigor by the soft rot of the feeding roots.

Field inoculations at Bloomington, Illinois, with 18 pedigreed strains of corn in the cool, wet spring of 1924 seem to confirm these results. The average reduction in stand was 30 per cent. The maximum reduction in yield of sound corn per plant was 65 per cent in one strain, notwithstanding thinning due to a 31 per cent reduction in stand. (Cooperative investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, the Wisconsin Agricultural Experiment Station, and the Funk Brothers' Seed Company.)

Sclerospora graminicola on corn. I. E. MELHUS and FRANK VAN HALTERN.

The oospores of *Sclerospora graminicola* on *Setaria viridis* may cause seedling infection of corn, teosinte, sorghum, and millet. The symptoms produced on these hosts,

in general, resemble those on *Setaria viridis* in the open, except that oospores have not been observed on corn, teosinte, or sorghum. Conidial sporulation is confined to the early-seedling stage. This fact makes the disease difficult, for the present, to diagnose in the field on corn. Plants showing the characteristic etiolation, dwarfing, and striping were collected this past season on corn seedlings and more mature plants. Oospore infection has never been obtained after emergence of the seedling.

Yellow pearl popcorn inoculated in the greenhouse with oospores and transplanted to the field in the seedling stage showed 73 per cent infection. Sixty-five per cent of these died before maturity. The remaining plants were stunted, produced neither ears, ear shoots, nor normal tassels. Ninety-nine per cent of the inoculated checks lived.

The incubation period for oospore infection on *Setaria* was six days, teosinte eight, and corn ten. Oospore infection is prevailingly systemic. The oospores require no rest period. They may function immediately on liberation from the host tissues; yet they remain viable in the soil for at least two growing seasons. Oospores stored dry in the laboratory for 14 years were not viable.

Observations on the stem rot of rice caused by Sclerotium oryzae Catt. V. H. YOUNG.

Stem rot caused by *Sclerotium oryzae* is the most serious disease of rice in Arkansas. It is rather widespread and all commercial varieties are attacked. Observations on diseased fields, allowed to lie idle one season, indicate that the incidence of the disease has thereby been greatly decreased. Other hosts for the stem-rot fungus have not been found, but red rice, a weed variety, is susceptible and lives over, producing seed on unirrigated land and thus carrying the disease over the fallow period from one rice crop to the next. Diseased plants ordinarily do not appear in numbers until after the final drainage. However, diseased red rice has been found a month prior to harvest and the greatest losses observed were in fields containing such volunteer plants.

Infection of young plants was not observed, but seedling infections were secured with pure cultures *in vitro*. Soil inoculations at higher temperatures in soil temperature tanks did not result in earlier infection, nor was the *amount* of infection influenced by soil temperatures from 22.5° C. to 35° C. Soil from affected fields gave infection under greenhouse conditions. Growth of *Sclerotium oryzae* on rice stubbles after harvest results in considerable increase in the amount of over-wintering material.

Studies in rust resistance. (Invitation paper) E. B. MAINS.

Microscopical studies of rust resistance and susceptibility. (Invitation paper) RUTH F. ALLEN.

Smut resistance in cereals. (Invitation paper) GEORGE M. REED.

Susceptibility of wheat varieties and selections to loose smut. F. D. FROMME.

Differences in incidence of loose smut in the Stoner and Leap varieties of wheat are shown to be due to differences in susceptibility. Inoculation of florets of the two varieties produced 62 per cent of infected plants of Stoner and only 3 per cent of infected plants of Leap. Thirty-five per cent of Stoner heads were smutted as compared with 4 per cent of heads of Leap.

Other wheat varieties grown under test conditions during several seasons showed marked differences in incidence of loose smut and each variety, with few exceptions, showed a high degree of uniformity when rated in terms of a standard. Similar differences, but of greater degree, are found in pure line selections of Fulcaster. Some are very susceptible, some moderately so, and some are highly resistant. The most sus-

ceptible selection is eight times as susceptible as the Fulcaster parent and the most resistant is twenty times as resistant as Fulcaster. This variability is possibly related to the hybrid origin of the variety. The probability of developing a highly resistant strain of Fulcaster through pure line selection is indicated.

Breeding and selecting for resistance to corn root-rot. (Invitation paper) G. N. HOFFER.

Inheritance of immunity from Melampsora lini. A. W. HENRY.

Crosses were made between immune and susceptible varieties of *Linum usitatissimum.* Three immune parents were used; a selection from a lot of commercial Argentine flax; Bombay (C. I. 42); and Ottawa 770B. The F_1 plants of all crosses were immune. The segregation in the F_2 of a cross between the Argentine selection and Saginaw, a susceptible fiber variety, approximated a ratio of 15 immune plants to 1 susceptible plant. The F_2 of a cross between Bombay and Winona, a susceptible seed flax, showed a simple monohybrid segregation. Apparently there was a single factor difference between immunity and susceptibility in a cross between Ottawa 770B and Saginaw. The former has white flowers and yellow seeds, and the latter blue flowers and brown seeds. Blue flowers and brown seeds were dominant, and a ratio of 3 blue-flowered, brown-seeded plants to 1 white-flowered, yellow-seeded plant was obtained in the F_2. These characters apparently are linked and were inherited independently of immunity from rust, so that in the F_2 a segregation approximating a 9 : 3 : 3 : 1 ratio was obtained. (Cooperative investigation by the Minnesota Agricultural Experiment Station and the Office of Cereal Investigations and Fiber Plant Investigations, U. S. Department of Agriculture.)

Seed potato improvement work. (Invitation paper) WILLIAM STUART.

Studies upon the inheritance of Fusarium-resistance in cabbage. J. C. WALKER.

By selfing individual cabbage plants through three generations, a line has been established which is apparently homozygous for resistance to *Fusarium conglutinans* under such field conditions as favor development of the yellows disease. Similarly, through selfing and close-pollination, individuals, apparently homozygous for susceptibility under the same environment, have been selected from commercial varieties. The F_1 hybrids from several crosses between resistant and susceptible individuals were nearly all disease-free. A total of 1,135 F_2 hybrid plants were grown in 1925 on infested soil; 843 were completely free from yellows, 292 were diseased. Segregation was therefore close to a ratio of three resistant to one susceptible. Several F_1 plants were crossed with a susceptible individual. Of these F_2 hybrids, 151 plants were grown in infested soil; 77 were completely resistant and 74 were diseased. Segregation was close to the 1 : 1 ratio. These data indicate that resistance to yellows, as expressed under field environment, is a dominant unit-character. (Cooperative investigations between the Office of Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Wisconsin.)

Resistance in sugar beets to curly-top. EUBANKS CARSNER.

A large number of strains of sugar beets selected for their apparent resistance to curly-top, together with 16 strains selected only for desirable agronomic characters, were subjected to a rigorous test in a leafhopper (*Eutettix tenella*) infested locality. In addition to the usually great natural infection hazard, viruliferous leafhoppers were caged on each plant. Control plats of commercial seed were similarly treated.

The results showed that among the strains which had been selected without regard for resistance, some were more susceptible than the controls, while others, especially one strain, were less affected than the controls. Among the strains which had been selected for resistance several were definitely less injured than the controls—one of them conspicuously so.

A comparatively large planting of the first generation progeny of a mass selection for resistance also suffered less from the disease than did the commercial parent strain. The results of the work thus far, especially those of the past season, establish the fact that there are marked variations in resistance to curly-top among sugar beets, and seem to justify the belief that a satisfactorily resistant commercial strain can be developed.

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PHYSIOLOGIC SPECIALIZATION IN THE LEAF RUST OF WHEAT, PUCCINIA TRITICINA ERIKSS.[1]

E. B. MAINS AND H. S. JACKSON[2]

WITH PLATES VI TO IX

INTRODUCTION

A study of the leaf rust of wheat, caused by *Puccinia triticina* Erikss., was begun in 1918 in cooperation with the Office of Cereal Investigations, United States Department of Agriculture. The results obtained in a study of the life history of this rust have already been reported (13). As another phase of the investigation, a study has been made to determine the susceptibility of wheat varieties to leaf rust with the object of obtaining resistant varieties. In order to determine the value of the resistant varieties of wheat, it became necessary to make an intensive investigation to determine whether the rust, as it is found in this country, is homogeneous or whether it consists of a number of forms differing in their pathogenicity upon different varieties. The results obtained by Eriksson and Henning (7), Eriksson (5, 6), Hitchcock and Carleton (11), Carleton (2), Freeman and Johnson (8), and Jackson and Mains (13) indicate that the leaf rust of wheat is very closely restricted as to hosts. Wheat has been found to be the only favorable grass host for the uredinial and telial stages, and a few species of *Thalictrum* are the hosts of the aecial stage.

The question as to whether there exist in the leaf rust of wheat specialized forms distinguished by their parasitism, similar to those which have

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[2] The writers wish to thank Dr. C. R. Ball, Dr. H. B. Humphrey, and Dr. C. E. Leighty for criticisms and help in developing this study, and also the many plant pathologists and others who have furnished collections of leaf rust for this investigation. Thanks are also due Mr. Forest Fuller, Mr. Emile Mardfin, Mrs. Florence Smith Sutton and Mr. Leroy Compton for assistance in making inoculations.

been found by Stakman and coworkers (14, 23, 24) and Melchers and Parker (18) in connection with their studies on *Puccinia graminis tritici* Erikss. and Henn., has been unanswered up to the present. The investigations of the stem rust of wheat show that such strains are of great importance in breeding for stem-rust resistance, and that a knowledge of them is necessary before it is possible to draw conclusions as to the value of resistant varieties. This paper deals with the results obtained in a study of this problem as it relates to leaf rust of wheat and which have been reported in part before the American Phytopathological Society (16, 17).

METHODS

Collection of inoculum. When the leaf rust was collected locally, the method usually employed was to bring in rusted leaves of wheat and sow the spores at once on healthy seedlings. When inoculum was obtained from other localities, rusted leaves were placed in heavy manila envelopes and mailed to the laboratory at LaFayette, Ind. It was not found necessary to wrap the leaves in oiled paper to prevent drying; in fact, the germinability of the spores is better if oiled or moistened paper is not used for wrapping. It was found, also, that inoculum mailed without precautions against drying would retain its viability for a number of months (15) if placed in an ice-box. In this way it was possible to collect the rust at a number of places before harvest, store it during the hot summer months when it is difficult to culture the rust in the greenhouse, and then start cultures in the autumn.

Inoculation. A susceptible variety of wheat was used on which to maintain the rust cultures. At first Dawson Goldenchaff was used; later, Michigan Amber and Big Harvest Fultz were employed. Usually about ten plants were grown in a four-inch pot. Previous to inoculation all plants were kept in a small greenhouse, in which no rust culture was allowed, and were carried from there to another greenhouse where they were inoculated and studied. One of the difficulties encountered early in the course of the work was the development of powdery mildew of wheat, *Erysiphe graminis tritici* Marchal, which often made culture study difficult. If plants bearing stock cultures of the rust became infected with mildew, the mildew was sown with the rust and interfered with the study of the rust susceptibility of the wheat varieties. This difficulty was overcome in two ways. The hot-water heating pipes in the small greenhouse where uninoculated plants were grown were painted with a mixture of lime and sulphur. The sulphur fumes thus generated proved very effective in preventing the development of mildew. A study of wheat varieties also resulted in revealing several which were highly resistant to mildew. Among these was a strain of Michigan Amber which was also very susceptible to rust. The complications

caused by the mildew may be avoided by using this strain as the variety for stock cultures.

The wheat seedlings were inoculated when in about the second-leaf to fourth-leaf stage. This was done by first drawing the leaves between the moistened fingers, thus removing the waxy covering and leaving a film of water on the leaves. Spores were scraped from rusted wheat and spread on these wet leaves by means of a scalpel. When only one or two plants were inoculated, they were placed under a bell jar or plant cover. When a larger number were inoculated, they were placed in galvanized iron tanks. A high humidity (practically 100 per cent) was maintained by placing a layer of wet sphagnum in the bottom. The tanks were covered by a lid in which a pane of glass was inset to allow entrance of light and prevent etiolation of the wheat seedlings. The plants were kept in these inoculation chambers for 48 hours, being atomized with water morning and evening to help maintain the humidity and keep water on the wheat leaves. This is necessary because a high humidity and actual contact with condensed water are two of the conditions necessary for spore germination. Temperature also is an important factor. Temperatures between 16 and 20° C. are most favorable for the spore germination of leaf rust of wheat, while little germination takes place at 5° C. or below, and 30° C. or above. When the temperature of the greenhouse was too high for the best spore germination, as sometimes occurs in late spring, summer, or early fall, inoculation chambers were cooled to optimum conditions by keeping them sprayed with water by means of a system of spray nozzles attached to the greenhouse water supply. When the inoculated plants were removed, it was found advisable not to place them in full sunlight, as they were likely to be scalded. Consequently, they usually were taken from the inoculation chambers in the late afternoon.

As several different cultures of leaf rust were being studied at the same time, for possible differences in parasitism, it was necessary to take precautions to prevent contamination of one by the other. Consequently after sowing one culture and before sowing the next, all instruments and vessels were sterilized in alcohol or in cleaning fluid, and the hands thoroughly washed. The cultures were placed in separate incubation chambers and later removed to separate plant covers or tent-like chambers. The sides and ends of the chambers are of cypress and the roof of two panes of glass which slide up and down in grooves, thus permitting easy access to the culture with minimum exposure to wind-blown spores.

Uredinia of leaf rust usually appear in 6 to 10 days, depending upon conditions of light and temperature. After the first uredinia have appeared, others are often developed later in a circle around them. The culture continues to sporulate as long as the infected leaf remains alive,

which varies with the amount of rust, the available food supply of the leaf, and especially the environmental conditions affecting the latter. As conditions are not favorable for spore germination in the plant cover, humidity especially being low, reinfection does not occur, and it is necessary to resow the rust. This is usually done on fresh pot cultures of wheat seedlings. Such transfers are necessary about every 4 to 6 weeks.

From the cultures of rust maintained in the plant covers, sowings were made on pots of seedlings of various varieties of wheat. Inoculation was carried out as described above, with the exception that, when the sets of varieties were too large to place under plant cover after removal from the incubation chambers, they were placed on the greenhouse bench. Further inoculations with the cultures were always from the susceptible variety protected from contamination within the plant cover.

Pure lines of leaf rust. In the course of the investigation, several of the cultures gave evidence of being composed of more than one physiologic form of the rust. Consequently it became necessary to devise a method of obtaining pure cultures or pure lines of the rust, in order to be sure that the results obtained were really characteristic of the forms and not due to a mixture of two or more forms. The method finally used was to obtain cultures of the rust from single urediniospores.

Wheat was sown in four-inch flower pots and immediately covered with a lantern globe, the top of which was covered with a thin layer of cotton held in place by a piece of cheesecloth tied over the top. The lantern globe fitted down tightly into the soil of the pot; consequently there was practically no chance of the wheat seedlings which came up within becoming contaminated from outside spores. When these plants were large enough to inoculate, a rust-infected leaf was gently tapped over a Petri dish, so that ripe urediniospores were scattered over the bottom of the dish. This dish was then placed under the higher powers of a binocular microscope and a single isolated urediniospore located. This was picked up by means of a glass rod drawn to a fine point; the point was wet in water, and the spore usually adhered readily. This spore was then transferred to a leaf of one of the plants inside the lantern globe. Each of the plants in the globe was inoculated with a single spore. The inoculated plants were then carefully watched, and, with the appearance of the first uredinium, all the other plants under the globe were removed. When this uredinium reached maturity, urediniospores were sown by means of a scalpel upon other plants grown within a lantern globe, thus maintaining the culture. Several such cultures were started from single spores. The inoculum produced under such lantern globes, although limited, is sufficient to inoculate from six to ten pots of wheat seedlings. When larger numbers were to be inoculated,

the culture was first multiplied on the susceptible variety under a plant cover and from this the series was inoculated. However, the culture under the lantern globe was also continued, and, if further sets of varieties were to be inoculated, the rust was again multiplied and sown from this, thus insuring the purity of the culture.

In making single-spore cultures, a difficulty was encountered in the late fall and winter. During this period of low light intensity, the percentage of germination of urediniospores is usually rather low, oftentimes being only 10 per cent. It readily can be seen that, where only ten spores are sown, there is considerable chance that germinable spores will not be selected and thus no culture obtained. This frequently resulted, and the task of obtaining single-spore cultures proved a very laborious one, for it was necessary to inoculate with a large number of single spores. It was found, however, that if the Petri dish containing spores was covered and set aside in the laboratory for two or three days, the percentage of germinable spores increased greatly and at least one of the ten inoculated plants under the lantern globe usually became infected.

Another difficulty encountered is that of maintaining single-spore cultures during the late spring and summer months, when the temperature of the greenhouse naturally is high and especially so under the lantern globes. Such conditions do not favor the growth of wheat. Wheat under the lantern globes becomes infected also by a number of fungi, especially *Helminthosporium* spp., and as a result the plants are killed and many cultures lost. Also, during this period, the leaf rust of wheat is very prevalent in the field. In most seasons nearly all the varieties of wheat generally grown in the eastern United States are more or less heavily infected with leaf rust. Consequently during this period urediniospores are very prevalent in the air. Stakman *et al.* (22) report finding them as high as 10,000 feet. With the greenhouse ventilators necessarily open at this time of year, when single-spore cultures are carried on wheat, except under very confined conditions, they become contaminated by these wind-carried spores. It therefore has been very difficult to carry the cultures from one year to another. Since, however, it has been found that the urediniospores will retain their viability to some extent for several months, it is possible to collect the urediniospores of each culture, store them through the summer in vials in an icebox and start the culture anew in the autumn.[3] Although some cultures are lost, this method has made it possible to carry over from year to year a number of the cultures with a reasonable certainty that they were pure.

[3] The effect of drying on spore germination and the method of storing inoculum have been worked out in connection with studies upon spore germination being made by Mr. Leroy Compton under the direction of the writers.

The data which had been accumulated from a study of the susceptibility of wheat varieties to rust as collected in the field made it possible to select a set of varieties which would be useful to differentiate any strains which might be present. It was possible, also, when making single spore cultures, to select those which differed most widely in their effects on such varieties, thus obtaining a series of cultures which in general were found to be different specialized forms of the rust.

RESULTS

Types of reaction shown by wheat to leaf rust. As but few data were available on the susceptibility of wheat varieties to the leaf rust of wheat, it was first necessary to determine which of the many varieties and strains were resistant to this rust. Therefore approximately 300 varieties and strains of wheat were obtained in 1918 and studied for susceptibility to 10 cultures of leaf rust obtained from different parts of the country. Of these varieties, the most promising were selected for further study. During 1919, 1920, and 1921, data concerning the susceptibility of wheat varieties to leaf rust were obtained from various wheat nurseries, the most extensive being that of the Office of Cereal Investigations at Arlington Experimental Farm, Washington, D. C. Those varieties showing the least rust were studied for susceptibility to leaf rust in the greenhouse during the winter months. Cultures of rust from various parts of the country were obtained for this purpose. It is not proposed in this paper to give an account of the results obtained with all the wheats studied. However, 550 varieties or selections covering a wide range of wheats, including most of the principal American varieties of *Triticum aestivum* L., *T. compactum* Hort., *T. durum* Desf., *T. dicoccum* Schrb., *T. spelta* L., *T. turgidum* L., *T. polonicum* L., and *T. monococcum* L. were inoculated with leaf rust. One hundred and eighteen cultures of leaf rust were used. Naturally all of these cultures were not sown upon all of the varieties and selections. As the study progressed, the number of varieties of wheat was greatly reduced, only those showing more or less marked resistance to one or more of the cultures of the rust being retained.

One of the interesting facts developed in this study was the reaction of a number of varieties showing more or less resistance in the field. Thus, Kanred, which has been noted by Melchers and Parker (18) and by the writers (16) to be usually highly resistant under field conditions, has not shown in general a corresponding high resistance when inoculated in the seedling stage in the greenhouse. Thus, in 1920, Kanred showed only a trace to 10 per cent of rust in nurseries at Madison, Wis., and Washington, D. C. Again in 1921, Kanred showed only a trace to 35 per cent in nur-

series at Murfreesboro, Tenn., Brookings, S. D., Washington, D. C., La-Fayette, Ind., and Madison, Wis. Kanred was well exposed to infection, since a number of varieties nearby showed 75 to 100 per cent infection. Seedlings of Kanred, when inoculated with collections of rusts from each of these nurseries, were more or less susceptible, varying in reaction from 2 to 4 in the seedling stage in the greenhouse. Other varieties, including some of the durums and emmers, while having some resistance in the seedling condition in the greenhouse, were generally almost immune in the field. This was true especially with certain of the cultures. In general, the number of varieties found to be highly resistant in these greenhouse studies was relatively small.

In the course of this study several different kinds of host reaction were found in the varieties studied in the seedling stage in the greenhouse. These are grouped into five classes with five corresponding types of infection, designated by the symbols 0 to 4, as follows:

Classes of host reaction (See Plate VI)		Types of rust infection
0	Highly resistant	No uredinia formed; small flecks, chlorotic or necrotic areas more or less prevalent.
1	Very resistant	Uredinia few, small, always in small necrotic spots. Also more or less necrotic areas produced without development of uredinia.
2	Moderately resistant	Uredinia fairly abundant, of moderate size, always in necrotic or very chlorotic spots. Necrotic spots seldom without uredinia.
3	Moderately susceptible	Uredinia fairly abundant, of moderate size. No necrosis produced, but sometimes slight chlorosis immediately surrounding the uredinia.
4	Very susceptible	Uredinia abundant, large. No necrosis or chlorosis immediately surrounding the uredinia. Infected areas sometimes occurring as green islands surrounded in each case by a chlorotic ring.

Reaction of wheat varieties to physiologic forms. The first indication of the existence of physiologic forms of leaf rust of wheat was discovered through the inoculation of Malakoff, C. I. 4898, with a culture of rust from Murfreesboro, Tenn. Malakoff had hitherto shown a high degree of resistance to fifteen other cultures of leaf rust with which it had been inoculated. To the culture of rust from Murfreesboro, however, it was highly

susceptible. As other cultures were obtained, some were found to which this variety was resistant, while to others it was highly susceptible. In a similar way, the following were the principal varieties found to show differences in reaction to different cultures of the rust: Turkey (Malakoff), C. I. 4898[4]; Turkey 47 (received from Kansas labeled as Turkey × Bearded 47); Norka, C. I. 4377; Mediterranean, C. I. 3332; Mediterranean, C. I. 5776; Mediterranean (Imperial Amber), C. I. 4860; Hussar, C. I. 4843; Democrat, C. I. 3384; Beloglina, C. I. 1544; Beloglina, C. I. 2239; Pacific Bluestem, C. I. 4821; Banat, C. I. 4879; Hungarian, C. I. 4830; four unnamed spring wheats, C. I. Nos. 3747, 3756, 3778 and 3779; Webster, C. I. 3780; Peliss, C. I. 1584; Acme, C. I. 5284; Arnautka, C. I. 1493; Arnautka, Minn. 337; Monad, C. I. 3330; Mindum, C. I. 5296; Khapli emmer, C. I. 4013; Vernal emmer, S. D. 293; Bearded Winter spelt, C. I. 1724; White Winter spelt, C. I. 2968; Rivet, C. I. 4201; Titanic, C. I. 5535; Polish, C. I. 5524; Einkorn, C. I. 2433; and Double Einkorn "Double emmer" from Ottawa.

The identity of these varieties has been checked by Dr. C. E. Leighty, of the Office of Cereal Investigations. Descriptions of most of them are given by Clark, Martin and Ball (3) in their classification of American wheat varieties. As the unnamed or recently named varieties are not described in that publication, the following description of each has been kindly furnished by Dr. Leighty. They are introductions received from St. Petersburg, Russia, in 1912.

C. I. No. 3747. Plant spring habit; stem white; spike awned, fusiform, lax, speltoid; glumes glabrous, brownish, long, midwide, distinctly keeled throughout entire length; shoulders narrow, oblique; beaks 1 to 18 mm. long; awns 3 to 7 mm. long; kernels red, long, semihard.

C. I. No. 3756. Plant spring habit, midseason, midtall; stem white; spike awned, fusiform, lax, speltoid, inclined; glumes glabrous, white, midlong, wide, distinctly keeled throughout entire length; shoulders wide, elevated; beaks 8 to 20 mm. long; awns 3 to 8 mm. long; kernels red, long, hard.

C. I. No. 3778. Plant spring habit; stem white; spike awned, fusiform, lax, speltoid; glumes glabrous, brown, midlong, midwide; shoulders midwide, square; beaks 1 to 5 mm. long; awns 3 to 8 mm. long; kernels red, midlong, hard.

C. I. No. 3779. Plant spring habit; stem white; spike awned, fusiform, lax, speltoid; glumes glabrous, brown, long, midwide; shoulders midwide,

[4] Numbers preceded by "C. I." are the accession numbers of the Office of Cereal Investigations, U. S. Department of Agriculture. Those labeled "Minn." are of the Minnesota Agricultural Experiment Station. Those designated "S. D." are of the South Dakota Agricultural Experiment Station.

square; beaks 3 to 20 mm. long; awns 3 to 8 mm. long; kernels red, midlong to long, hard.

Webster, C. I. No. 3780. Plant spring habit, midseason, midtall; stem white, weak; spike awned, fusiform, lax, speltoid; glumes glabrous, brownish, long, narrow, distinctly keeled throughout entire length; shoulders narrow, oblique; beaks 2 to 10 mm. long; awns 3 to 8 mm. long; kernels red, long, hard.

In the course of these studies it was found that oftentimes the culture of leaf rust, as obtained from the field, produced two or more quite distinct types of infection on one or more of the varieties inoculated. In order to determine whether this represented a variation in the reaction of the variety or was due to a mixture of two or more physiologic forms, these varieties were again inoculated with the rust. If more than one form was present, there would be a rapid multiplication of the one to which the variety was susceptible, while there would be little or no multiplication of the strain to which the variety was resistant. The same set of varieties was again inoculated with the same rust and the results compared.

The results obtained from the study of a culture received from Mr. C. O. Johnston, of Manhattan, Kansas, well illustrates the condition found in field collections. This culture severely infected Malakoff, C. I. 4898, producing a type 4 infection; whereas Mediterranean, C. I. 3332, showed several necrotic spots containing occasionally a small uredinium. Associated with these, however, were a few well developed uredinia which were accompanied neither by necrosis nor chlorosis. Urediniospores from these well developed uredinia were placed on plants of Mediterranean, C. I. 3332, where they produced abundant infection of type 4. The rust as developed on Mediterranean was then used as inoculum on Malakoff, C. I. 4898, and Mediterranean, C. I. 3332. The latter showed a type 4 infection, while Malakoff showed only a slight flecking (type 0), no uredinia being developed. The original culture of rust was then sown on Malakoff, and the rust obtained from this variety was transferred to Malakoff and Mediterranean. As a result, Malakoff was heavily rusted, showing type 4 infection. Mediterranean, on the other hand, showed considerable necrosis with an occasional small uredinium (type 1). Apparently the original culture consisted of at least two forms, one much more prevalent than the other.

In a somewhat similar manner, a culture obtained from aecia of the leaf rust of wheat on *Thalictrum flavum*[5] L. produced heavy infection on Mala-

[5] The aecia from which this culture was made were obtained by inoculating *Thalictrum flavum* in the greenhouse with wintered teliospores of *Puccinia triticina* from Clemson College, S. C. Aeciospores were collected from a number of leaves of *Thalictrum* and transferred to wheat (Michigan Amber) from which uredinia subsequently were obtained. The culture thus developed on wheat was sown as indicated.

koff, C. I. 4898, and the spring wheats, C. I. 3756, and Webster, C. I. 3780. Each of these varieties was reinoculated with the same rust. The three cultures of rust thus established were each transferred back to the same three varieties. The culture carried on the variety Malakoff was then found to infect this variety heavily, while it produced only slight flecking on varieties C. I. 3756 and Webster. The culture carried on C. I. 3756 produced heavy infection on both C. I. 3756 and Webster, whereas Malakoff was only slightly flecked. The culture carried on Webster produced heavy infection on Webster and 3756, while Malakoff was only slightly flecked. In this case there was no indication in the original culture that more than one form existed, the two forms apparently being about equally prevalent in the mixture and infecting all three varieties so heavily that resistance as indicated by the slight flecking was obscured. The cultures thus isolated remained uniform for their actions on the varieties named above. Other examples might be given, but these are sufficient to show that mixtures of forms apparently often exist in cultures of leaf rust as obtained in the field.

While such mixtures may be immediately evident when cultured, as in the first example given above, in other cases, as in the second example, the mixture may not be suspected until after culturing and cross inoculating on a series of varieties. Attempts to isolate forms by such a method proved very unsatisfactory. In such mixtures, where varieties were susceptible to one form and only moderately resistant to the other, urediniospores might be produced by both, and the two forms of rust would still be continued when the rust was again used as inoculum. In these cases it was difficult to decide whether there really was a mixture or whether the results were due to the reaction of the variety to a single form of the rust. Even when two quite different cultures were separated, it was by no means certain that each of these might not also be a mixture of two or more physiologic forms. With the increasing number of varieties which could be used as differentials, the number of inoculations and cross inoculations necessary to purify cultures made this method prohibitive.

These experiences demonstrated that it was necessary to be sure that pure lines of the rust were being studied. So it was decided that a study of the physiologic forms present in the leaf rust of wheat must be based only on cultures which had been started from a single spore. The method used has been described above. During the winter of 1921–1922, fourteen cultures from single spores were obtained and studied on the varieties mentioned above which had previously given promise of being differential for the physiologic forms of the rust. The previous study of the rust cultures had indicated those which would most certainly produce distinct physiologic forms, and the cultures from single spores were made from these. This resulted in the finding of a number of distinct forms.

During the winter of 1921–1922, 129 varieties and strains of common wheat were studied for their susceptibility to from 4 to 12 single-spore cultures of leaf rust. These included most of the varieties which had been noted as less heavily rusted in the field as well as those noted as resistant in the greenhouse studies of previous years. Most of these proved to be susceptible to all the cultures used. In a few strains of Michigan Amber, Fultz, Fulcaster, Theiss, Red May, Beloglina, and Banat, a few outstandingly resistant seedlings occurred. These were grown to maturity and are giving promise of being valuable resistant lines of these varieties.

Several of the varieties which in previous years had shown marked resistance in the greenhouse were again highly resistant to part of the cultures in 1921–1922, except for a few susceptible seedlings. These were Mediterranean (Imperial Amber), C. I. 4860; Beloglina, C. I. 1544; Beloglina, C. I. 2239; Pacific Bluestem, C. I. 4821; Banat, C. I. 4879; and Hungarian, C. I. 4830. The reaction of Mediterranean, C. I. 5776 was very similar to that of Mediterranean, C. I. 3332 except for what was apparently due to a slight mixture by another variety. On account of these impurities, it was not considered desirable to continue to use these varieties in the study of physiologic forms until purified for rust resistance.

During the winter of 1921–1922, those varieties of the durums, emmers, spelts and einkorns which had previously shown some promise were studied for susceptibility to 12 single-spore cultures belonging to 10 different physiologic forms. Only two of the wheat varieties belonging to these groups, Einkorn, C. I. 2433, and Vernal emmer, S. D. 293, were comparable for resistance to the varieties listed in table 1. High resistance to certain cultures was noted in some of the varieties, especially Einkorn, C. I. 2433 and Vernal emmer, S. D. 293, the latter being resistant to some degree to all forms. In general, however, resistance was only moderate (type 2) and in many cases it was rather intermediate between types 2 and 3, well developed large and small uredinia being produced with and without either necrosis or chlorosis all on the same leaf, an infection comparable to the X type of Stakman and Levine (23). While high susceptibility to a number of the cultures was shown by some of the varieties, there were usually, although not always, some indications of chlorosis.

Since common bread wheats have been found which sharply differentiate the physiologic forms, the above varieties have not been used to any great extent in these studies. It is of interest to note, however, that while most of these varieties have been only moderately resistant or susceptible in these greenhouse studies, they have shown high resistance in the field. Observations during the period 1919 to 1924 inclusive, especially at LaFayette, Ind.; Washington, D. C.; Knoxville, Tenn.; Experiment, Ga.; and Madison, Wis., show that the varieties of durums, emmers, and einkorn listed above

have been highly resistant in the field, usually showing only flecking with the development of a few small uredinia. In a few instances, especially in the case of Khapli, there has developed 35 to 50 per cent of rust. In the seedling stage in the greenhouse, however, they have shown no such uniform rust resistance except in the two instances mentioned above.

Eleven varieties of common wheat showed marked resistance to some of the single-spore cultures. These are Malakoff, C. I. 4898; Turkey 47; Norka, C. I. 4377; Mediterranean, C. I. 3332; Hussar, C. I. 4843; Democrat, C. I. 3384; Webster, C. I. 3780; and the unnamed spring varieties, C. I. Nos. 3756, 3778, 3747 and 3779. By the use of these, the cultures of leaf rust easily could be separated, as they were for the most part either highly resistant or highly susceptible to the single-spore cultures. While a number of the other varieties mentioned could be used, these eleven give more clear-cut differentiations. As they are common wheats, the group in which leaf rust resistance is of the greatest importance, it was decided to use this set for the time being in a study of physiologic forms.

Some additional single-spore cultures were made and studied during the winters of 1922–1923 and 1923–1924, and two additional physiologic forms were distinguished and more information gathered concerning the others.

The susceptibility of the varieties employed, and the characteristics of the physiologic forms of the rust so far separated, are given in table 1. To designate the various physiologic forms differentiated, Arabic numerals are employed to conform to the usage adopted by the American Phytopathological Society in January, 1925.[6] The different forms may be separated by a key, which is given in table 2. Under the various physiologic forms in table 3 are listed the single-spore cultures according to the locality from which the original collection was obtained.

Each of these 33 single-spore cultures was sown at least two different times on 7 to 15 seedlings of each variety. Therefore at least 14 to 30 seedlings of each of the 11 varieties were inoculated with urediniospores from each culture. Certain of these cultures which have been continued for some time and have been used in other studies have furnished inoculum for a much greater number. Thus certain of the single-spore cultures belonging to physiologic forms 3, 5, and 9 have been transferred to each variety from 9 to 13 different times, involving a total of between 255 to 320 seedlings of each of the 11 differential varieties for each of these single-spore cultures.

No difficulty was experienced in obtaining infection and, except for a very few plants, accidental mixtures, all the seedlings showed the character-

[6] See Report of Joint Committee on Nomenclature. Phytopathology 15: 316–317. 1925.

TABLE 1.—*Types of infection produced by twelve physiologic forms of the leaf rust of wheat, Puccinia triticina, on eleven differential strains of wheat*

Variety	Types of infection produced by physiologic forms											
	1	2	3	4	5	6	7	8	9	10	11	12
Malakoff, C. I. 4898	0	0–1	0–1	0–1	4	4	4	4	4	4	0	0
Turkey, 47	0	0–1	0–1	0–1	4	4	4	4	4	4	0	0
Norka, C. I. 4377	0	0–1	0–1	0–1	4	4	4	4	4	4	0	0
Unnamed, C. I. 3756	0	0–1	0–2	4	0	2	1–2	1	1–2	4	2+	4
do, C. I. 3778	0	0–1	2–2+[a]	4	0–1	4	1	4	1–2	4	3–4	4
Webster, C. I. 3780	0	0	0–2	1	0–1	1–2	4	4	4	4	1–2+	1
Unnamed, C. I. 3747	0	0	0–2	1–2	0–1	1	4	4	4	3–4	1–2+	0–1
do, C. I. 3779	0	0–1	2+[a]–4	3–4	0–1	3	1	4	4	4	3–4	4
Mediterranean, C. I. 3332	1	4	3–4	3–4	4	3–4	4	3–4	0–1	1–2	1–2	4
Hussar, C. I. 4843	1	3–4	0–1	2–2+	0–2+	3	1	1	1–2+	1–2	0–2	4
Democrat, C. I. 3384	0	4	4	1–2	4	3–4	4	1	0–1	1–2	0–2	4

[a] An infection type of 2+ is rather indeterminate. Uredinia are fairly abundant and large accompanied by pronounced chlorosis and sometimes some necrosis.

istic reaction of the variety as given in table 1 for the physiologic form in question. There was considerable difference between varieties in the amount of variation in reaction. Some varieties, such as Malakoff, Turkey 47, or Norka, showed very little. To physiologic forms to which these varieties were highly resistant, they showed uniformly a very high resistance under all conditions of inoculation. On the other hand, to some of the physiologic forms, some varieties, as for example Hussar, showed considerable variation, often being highly resistant when inoculated in the fall or winter and only moderately or slightly resistant when inoculated in late spring, indicating an environmental effect upon the reaction. These have been included in the range of susceptibility in table 1. With the study of additional cultures, it may be desirable to modify the characterizations of some of the physiologic forms to include cultures which may show but slight deviations. This is especially true of such forms as have been isolated but once and lost after but comparatively little study, including forms 1, 7, 8, and 12.

The reaction of these varieties of wheat to the physiologic forms is interesting because of the marked differences which were found to occur. The varieties Malakoff, C. I. 4898, Norka, C. I. 4377, and Turkey 47 sharply differentiate forms 1, 2, 3, 4, 11 and 12 from 5, 6, 7, 8, 9 and 10, being very highly resistant (types 0 or 1) to the first group and highly susceptible (type 4) to the second (Plate VII, A and C). These three varieties not only have agreed with each other in the type of infection produced by each of the 33 single-spore cultures belonging to the 12 physiologic forms, but also have reacted very similarly when inoculated with 118 bulk cultures obtained directly from the field. Malakoff is considered by Clark, Martin and Ball (3) to be synonymous with Turkey. This particular strain of Malakoff, bearing the C. I. accession number 4898, was derived from a single head selected by Dr. C. E. Leighty at the Kentucky Agricultural Experiment Station in 1913. Turkey 47 is a strain of Turkey obtained from the Kansas Agricultural Experiment Station. Apparently, it originated from a hybrid of Turkey with some other bearded wheat, as it was designated Turkey × Bearded. The strain, however, has all the characteristics of Turkey. "Norka, C. I. 4377, originated from a pure-line selection of a common wheat made from a plot of Kubanka durum wheat in 1908 by W. G. Shelley at Akron, Colo." The Turkey lines are hardy winter wheats with white glumes, while Norka is a spring variety with brown glumes. As far as the identification of physiologic forms of *P. triticina* so far studied is concerned, Malakoff, C. I. 4898, Turkey 47, and Norka, C. I. 4377, in reality constitute but a single differential group.

In this connection, it is of interest to note the susceptibility shown by a number of strains of Turkey (Plate IX, B). Five cultures of rust to

which Malakoff, C. I. 4898, is resistant, were transferred to another strain of Turkey received from the Kansas Agricultural Experiment Station under the name of Malakoff, Minn. 1479. This strain proved highly susceptible to all 5 of these cultures. In addition, 15 other strains of Turkey were inoculated with from 3 to 5 cultures of leaf rust to which Malakoff, C. I. 4898, is resistant. These were received under the names of Crimean, Minn. 832; Crimean, C. I. 1436; Hungarian, C. I. 5967; Kharkof, C. I. 4897; Kharkof, Minn. 1474; Kharkof, Tex. 583; Kharkof, Tex. 1500; Theiss, C. I. 4883; Theiss, C I. 5741; Turkey, Minn. 829; Turkey (Alberta Red), Minn. 1488; Turkey, Minn. 1548; Turkey, Minn. 1549; Turkey, Tex. 1497; and Turkey, C. I. 4884. All of these strains of Turkey proved more or less susceptible (type 3 or 4). They also were susceptible to a number of other cultures to which Malakoff, C. I. 4898, was susceptible. In fact, they were susceptible to all the cultures with which they were inoculated.

It is therefore evident that while two strains of Turkey—Malakoff, C. I. 4898, and Turkey 47—behave similarly, the variety Turkey is not uniform

TABLE 2.—*Key to the twelve physiologic forms of the leaf rust of wheat, Puccinia triticina*

Host and reaction	*Form No.*
Malakoff, C. I. 4898, resistant[a]	
C. I. 3756, resistant	
Mediterranean, C. I. 3332, resistant	1
Mediterranean, C. I. 3332, susceptible	
Hussar, C. I. 4843, susceptible	2
Hussar, C. I. 4843, resistant	3
C. I. 3756, susceptible[b]	
Mediterranean, C. I. 3332, resistant	11
Mediterranean, C. I. 3332, susceptible	
Democrat, C. I. 3384, susceptible	12
Democrat, C. I. 3384, resistant	4
Malakoff, C. I. 4898, susceptible[a]	
C. I. 3756, susceptible	10
C. I. 3756, resistant	
Mediterranean, C. I. 3332, resistant	9
Mediterranean, C. I. 3332, susceptible	
Webster, C. I. 3780, susceptible	
Democrat, C. I. 3384, susceptible	7
Democrat, C. I. 3384, resistant	8
Webster, C. I. 3780, resistant	
Hussar, C. I. 4843, resistant	5
Hussar, C. I. 4843, susceptible	6

[a] Types of infection 0, 1 and 2 are included here under the term resistant. Types of infection 3 and 4 are included under the term susceptible.

[b] Physiologic form 11 is included here, although C. I. 3756 shows a rather indeterminate type between 2 and 3. See table 1.

so far as its leaf rust susceptibility is concerned. It is also of interest that, although the strains of Turkey wheat are not similar in their behavior, a distinct variety, Norka, C. I. 4377, should agree so closely in its behavior with two of the strains of Turkey, Malakoff, C. I. 4898, and Turkey 47 (Plate VII, A and C, Plate IX, B, 2 and 3).

A somewhat similar situation exists in the variety Mediterranean. Mediterranean, C. I. 3332, is resistant to physiologic forms 1, 9, 10 and 11, and susceptible to the other eight (Plate VIII, B). This variety, however, is not so clear cut in its susceptibility to some forms as is Malakoff. When resistant, it may be only moderately so (type 2), as, for example, to forms 10 and 11; and oftentimes when it is susceptible to certain forms it is only moderately susceptible to them. While many uredinia are produced, they may be more or less accompanied by chlorosis as in the case of forms 3, 4, 6 and 8. Another strain of the variety Mediterranean, C. I. 5776, behaved very similarly to C. I. 3332. It apparently was not quite pure. Other strains of Mediterranean—C. I. 1395, C. I. 5614, and C. I. 5834—and Missouri Bluestem, C. I. 1912, showed themselves highly susceptible to cultures of the physiologic forms 1, 9 and 10 (Plate IX, A). It would appear, therefore, that within this agronomic variety there also exist different strains distinguishable by differences in their reaction to leaf rust.

Hussar, C. I. 4843, is resistant to 9 of the 12 forms, namely, 1, 3, 4, 5, 7, 8, 9, 10 and 11 (Plate VIII, C). Although it is resistant to a larger number of the physiologic forms than Malakoff, C. I. 4898, it is not always so highly resistant as the latter; the type of infection often approaches type 3, as in the case of forms 4, 5 and 9. On the other hand, this variety is often only moderately susceptible to the two forms, 2 and 6, and is most susceptible to form 12. Democrat, C. I. 3384, is resistant to six forms, 1, 4, 8, 9, 10 and 11 (Plate VII, D). It is also not quite so definite in some cases in its susceptibility and resistance as Malakoff.

The unnamed spring variety, C. I. 3756, is resistant to eight forms, 1, 2, 3, 5, 6, 7, 8, and 9. To form 11 it shows an intermediate reaction. To the remaining three forms, 4, 10, and 12, it is highly susceptible. The variety C. I. 3778 behaves very similarly to C. I. 3756 except that it is susceptible to forms 6 and 8 and more susceptible to forms 3 and 11 (Plate VIII, D). The spring variety, Webster, is resistant to eight forms, 1, 2, 3, 4, 5, 6, 11, and 12 (Plate VII, B). To four forms, 7, 8, 9, and 10, it is highly susceptible. The unnamed spring variety, C. I. 3747, has behaved very similarly to Webster, and the two may be considered as one differential group.

The unnamed spring variety, C. I. 3779, is resistant to four forms, 1, 2, 5 and 7. Its reaction to leaf rust, however, is not as pronounced in some instances as is the case with others of the differential varieties. Although

showing signs of resistance to form 3 (Plate III, A 1), in that some necrosis occurs, moderately large uredinia are produced in large numbers, many being accompanied by only a slight chlorosis. This differs from type 3 infection in the occurrence of necrotic areas and apparently resembles the indeterminate type of infection designated "X" by Stakman and Levine (23) for the stem rust of wheat.

TABLE 3.—*Localities from which the various physiologic forms of leaf rust of wheat have been obtained*

Physiologic forms	Number of cultures studied	Source and date
1	1	Pullman, Wash., 1921.
2	2	Murfreesboro, Tenn., 1921; Washington, D. C., 1923.
3	5	Manhattan, Kans., 1921, 1922; Jefferson, Ga., 1922; Washington, D. C., 1923; Experiment, Ga., 1924.
4	2	St. Paul, Minn., 1921; Pullman, Wash., 1921.
5	2	Jefferson Co., Kans., 1920; Knoxville, Tenn., 1922.
6	3	Madison, Wis., 1922, 1923; LaFayette, Ind., 1923.
7	1	Washington, D. C., 1921.
8	1	Murfreesboro, Tenn., 1921.
9	6	Raleigh, N. C., 1920; Ames, Iowa, 1921; Manhattan, Kans., 1922 (4 cultures).
10	8	Brookings, S. D., 1921; Jefferson Co., Kans., 1920; Madison, Wis., 1921; St. Paul, Minn., 1923; Bloomington, Ill., 1923; Lexington, Ky., 1921; Blacksburg, Va., 1921; Washington, D. C., 1923.
11	1	Berkeley, Calif., 1923.
12	1	Madison, Wis., 1922.

The order of the differential varieties, if listed according to the number of forms of rust to which they show resistance, would be: Hussar, C. I. 4843, resistant to nine; Webster, C. I. 3747 and C. I. 3756, each resistant to eight; Malakoff, C. I. 4898, Norka, C. I. 4377, Turkey 47, Democrat, C. I. 3387, and C. I. 3778, each resistant to six; C. I. 3779 and Mediterranean, C. I. 3332, each resistant to four. Malakoff, Norka, and Turkey 47 were outstanding for their uniform high resistance to all of the six forms to which they showed resistance.

Distribution of physiologic forms. Whether there is any regularity or fixity in the distribution of the physiologic forms is a matter of considerable importance, especially in connection with breeding for resistance. Most of our efforts have been directed towards discovering physiologic forms of the

rust and the varieties of wheat by which they may be differentiated. The time and labor involved in starting cultures from single spores, in order to make certain of purity of cultures, has limited the number of cultures which could be studied. While the 33 cultures thus studied cannot be considered as giving complete data on the distribution of the forms, taken with other data they afford some idea of the situation. Table 3 gives the localities from which the various physiologic forms of rust have been isolated. While form 10 is apparently the most prevalent, there is seemingly no sharp geographic limitation to the distribution of the various forms in the eastern United States. Too few single-spore cultures from the west have been studied to allow drawing any conclusions as to the apparent absence of such forms as 10 and 9 from that part of the country.

TABLE 4.—*Reaction of wheat varieties to original culture of leaf rust collected at Pullman, Washington, and to the single-spore cultures isolated from it*

Culture	Types of infection on										
	Malakoff C. I. 4898	Turkey 47	Norka C. I. 4377	C. I. 3756	C. I. 3778	Webster C. I. 3780	C. I. 3747	C. I. 3779	Mediterranean C. I. 3352	Hussar C. I. 4843	Democrat C. I. 3384
Original culture	0(4)[a]	0(4)[a]	0(4)[a]	4	4	3(0)[a]	1	4	0(4)[a]	1–2	0–1
Single-spore culture 1 (Physiologic form 1)	0	0	0	0	0	0	0	0	1	1	0
Single-spore culture 2 (Physiologic form 4)	0–1	1	1	4	4	1	2	3	4	2+	1

[a] A mixture of two types of infection, that represented by the number in parentheses being much less abundant.

Still further light is thrown upon this situation when the single-spore cultures are compared with the cultures from which they are isolated. In only a few cases, where both the original culture as obtained in the field and the single-spore cultures isolated and developed from it were studied, did they agree entirely in their behavior. This indicates that the physiologic forms of the leaf rust of wheat are quite generally mixed in the field.

While in most cases no attempt was made to isolate all the forms to be found at any one place, in a few cases a number of single-spore cultures were made which showed definitely the presence of several forms in the same locality. Thus from a collection of leaf rust made by Dr. H. B. Humphrey

at Pullman, Washington, on July 25, 1921, two single-spore cultures were obtained which differed markedly from each other, as is shown in table 4. It is to be noted, however, that the original culture not only showed evidence of this mixture of forms, but apparently a third physiologic form was present to which Malakoff was susceptible. This may have been form 10 and apparently made up only a small part of the mixture.

Another instance of mixture of physiologic forms has been demonstrated in the study of cultures from single spores isolated from a collection of rust received from Prof. L. E. Melchers from Jefferson Co., Kansas, in 1920. While the original culture was not studied on the full set of varieties, the results from those varieties inoculated showed evidence (table 5) that the original culture was a mixture. From this original culture two cultures were derived by continuing to transfer the rust to the varieties Malakoff and C. I. 3756. From each of the cultures thus developed, another was started from a single spore. The results of the study of these two cultures are given in table 5 and indicate that the original culture was a mixture of physiologic forms 5 and 10.

TABLE 5.—*Reaction of wheat varieties to original culture of leaf rust collected in Jefferson Co., Kansas, and to the single-spore cultures isolated from it*

Culture	Types of infection on										
	Malakoff C. I. 4898	Turkey 47	Norka C. I. 4377	C. I. 3756	C. I. 3778	Webster C. I. 3780	C. I. 3747	C. I. 3779	Mediterranean C. I. 3332	Hussar C. I. 4843	Democrat C. I. 3384
Original culture	4	...	...	0,4[a]	0,4[a]	0,4[a]	...	0,4[a]	...	...	...
Single-spore culture 1 (Physiologic form 5)	4	4	4	0	0–1	0	0–1	0	4	1	4
Single-spore culture 2 (Physiologic form 10)	4	4	4	4	4	4	4	4	2	1	2

[a] A mixture of types of reaction in about equal proportions.

In 1921, leaf rust, then being used by Mr. C. O. Johnston in his greenhouse studies at Manhattan, Kansas, was received for study. A culture from a single spore isolated from this material differed markedly from the original, as is shown in table 6. The next year Mr. Johnston again sent a culture of the leaf rust which he had been maintaining in the meantime in

the greenhouse. A study of this again gave nearly the same results except that Mediterranean and Democrat showed only a few well developed uredinia where there had been a moderate number before. A culture from a single spore (Culture 1) derived from the susceptible type on Mediterranean was developed and studied as well as four other cultures from single spores (Cultures 2–5) from the rust on Malakoff. The four latter all agreed

TABLE 6.—*Reaction of wheat varieties to original cultures of leaf rust collected at Manhattan, Kansas, and to single-spore cultures isolated from it*

Culture	Types of infection on										
	Malakoff C. I. 4898	Turkey 47	Norka C. I. 4377	C. I. 3756	C. I. 3778	Webster C. I. 3780	C. I. 3747	C. I. 3779	Mediterranean C. I. 3332	Hussar C. I. 4843	Democrat C. I. 3384
Original culture, 1921	4	4	4	2	2	4	4	4	4,1[a]	3	4,0[a]
Single-spore culture Physiologic form 3...	1	1	1	2	2	1	1	4	3	1	4
Original culture, 1922...	4	4	4	2	2	3–4	3–4	4	1(4)[b]	3	0(4)[b]
Single-spore culture 1 Physiologic form 3...	0	0–1	0–1	1	2	0	0–1	2+	4	0–1	4
Single-spore culture 2 Physiologic form 9...	4	4	4	2	2	4	4	4	0	2+	0–1
Single-spore culture 3 Physiologic form 9...	4	4	4	2	2	4	4	4	1	2+	1
Single-spore culture 4 Physiologic form 9...	4	4	4	2	2	4	4	4	1	2+	1
Single-spore culture 5 Physiologic form 9...	4	4	4	2	2	4	4	4	1	2+	0–1

[a] A mixture of two types of infection in about equal proportions.

[b] A mixture of two types of infection, that represented by the number in parentheses being much less abundant.

very closely with each other, proving to be physiologic form 9, but differed markedly from that obtained from Mediterranean, which proved to be form 3. The latter agreed very closely with the single-spore culture obtained in 1921. Apparently Mr. Johnston's original culture consisted of a mixture of forms 3 and 9, probably in nearly the same proportion. After being carried

for a year in the greenhouse, form 3 apparently became reduced and made up only a small part of the mixture.

While it has been impossible to study more cultures derived from single spores, some additional evidence as to the mixture of physiologic forms has been accumulated. Some data obtained in the earlier part of the study and which made evident the necessity of deriving cultures from single spores already have been partly discussed. In addition to this, rust was collected in 1923 on a number of the differential varieties in several different nurseries to determine if the rust in the different localities was homogeneous. The results of this study are given in table 7. It would appear that the rust in all three localities was not uniform. At least two and probably more physiologic forms were present at each place. It is interesting to note that, both at Knoxville and LaFayette, the rust from Mediterranean, C. I. 3332, did not infect Malakoff when inoculated in the greenhouse, while the rust from Malakoff infected both hosts. Apparently, also, the rust on the varieties C. I. 3747 and Webster at LaFayette was not well adapted to these varieties, for they proved highly resistant when inoculated with the rust from the same variety in the field.

There is some evidence that the physiologic forms of leaf rust present in any given locality may vary from year to year. While it has not been possible to accurately determine the exact identity of these forms from studies of single-spore cultures, a study of the susceptibility of varieties in the field for several years and testing of the rust cultures in the greenhouse indicate that such variations occur. No attempt will be made here to give all the data, a few examples being sufficient.

The variety Malakoff showed only a trace to 5 per cent of rust in the varietal nursery at LaFayette in 1919. To cultures of the rust collected that summer and studied during the winter in the greenhouse, Malakoff showed only a slight flecking, agreeing with the field evidence and indicating that physiologic forms of leaf rust to which Malakoff is susceptible were either scarce or lacking at LaFayette in 1919. In 1920, Malakoff showed 50 to 65 per cent of rust in nurseries; and studies conducted during the following winter in the greenhouse indicated that two physiologic forms were present, this variety being highly susceptible to one culture and resistant to the other. While not especially studied in the greenhouse the two following years, the host reaction in the field and the results shown in table 7 for 1923 indicate that two or more physiologic forms were again present, Malakoff being susceptible to at least one form and resistant to another.

There is also some evidence that, while physiologic forms to which Malakoff is susceptible may be present over a wide territory (table 3), they

develop best in the hard winter and spring wheat areas of the United States, where they are more frequently isolated than in the south and east. Thus in the territory comprising the States of New York, Pennsylvania, District of Columbia, Virginia, North Carolina, South Carolina, Georgia, Alabama, West Virginia and Mississippi, 29 collections of leaf rust have produced no infection on Malakoff, 23 produced heavy infection, and 9 were very evident mixtures. In the territory comprising the States of Tennessee, Kentucky, Indiana, Michigan and Illinois, only 7 collections produced no infection on Malakoff, 28 infected that variety heavily and 7 were evident mixtures. In the territory including the States of Wisconsin, Minnesota, South Dakota, Iowa, Kansas, Oklahoma, Arkansas and Missouri, only 3 collections pro-

TABLE 7.—*Reaction on wheat varieties caused by cultures of the leaf rust of wheat obtained from different varieties in the field*

Location of Varietal Nursery	Variety of wheat from which culture was obtained	Percentage[a] of field susceptibility of variety from which culture was obtained	Types of infection on					
			Malakoff C. I. 4898	C. I. 3756	Webster C. I. 3780	C. I. 3779	Mediterranean C. I. 3332	Democrat C. I. 3384
Washington, D. C.	C. I. 3756 Webster	100	0	4	1	4, 1[b]	4	4
	C. I. 3780 Mediterranean	50	0	4	2	4	4	4
	C. I. 4860	100	4	4	0	4	4, 1[b]	1
Knoxville, Tenn.	Malakoff	trace—						
	C. I. 4898 Norka	25	4	3	0	4	4	2, 4[b]
	C. I. 4377 Mediterranean	50	4	3	0	4	4	4
	C. I. 3332	100	0	0	0	4	4	4
LaFayette, Ind.	Malakoff							
	C. I. 4898	35–65	4	1	1	2	4, 1	
	C. I. 3756 Webster	75–100	4, 1[b]	3	1	4	4	
	C. I. 3780	5–25	3	1(4)[c]	1	3	4	
	C. I. 3747 Mediterranean	5–25	3	1	1	4	3, 1[b]	
	C. I. 3332 Hussar	75–100	0	2	0	3	4	
	C. I. 4843	25–50	3(1)[c]	1	0	4	4	4

[a] Based upon scale used in the Office of Cereal Investigations.

[b] Approximately equal mixture of types indicating mixture of physiologic forms.

[c] Mixture of types, that in parentheses being less abundant, indicating unequal mixture of physiologic forms.

duced no infection on Malakoff, 22 infected that variety heavily, and 5 were evident mixtures. From Arizona, Utah, Nevada, Montana, Idaho, Washington, Oregon and California, 11 collections caused no infection on Malakoff, only one infected Malakoff heavily, whereas 3 evidently were mixtures. Although physiologic forms to which Malakoff is resistant occur in the second and third groups of states, yet it is apparently in this region that those forms to which Malakoff is susceptible are the most prevalent, decreasing eastward, southward, and especially westward.

DISCUSSION

The results of this investigation show that the leaf rust of wheat consists of a number of physiologic forms distinguished by differences in their ability to parasitize varieties of wheat. A situation thus exists in this rust similar to that reported by Stakman and Levine (23) for the stem rust of wheat. In both cases these physiologic forms show but little morphologic basis for distinctions and are best identified by their effect when transferred to certain varieties of wheat.

Twelve physiologic forms of the leaf rust of wheat have been isolated thus far and distinguished by using 11 strains of wheat, comprising eight differential groups. Several of these forms of rust differ markedly from each other. For example, while all the eleven varieties are resistant to form 1, only three are resistant to form 10. Vernal emmer, S. D. 293, is the most outstanding of the varieties for resistance in the seedling stage in greenhouse studies, being more or less resistant to all of the cultures sown upon it. Unfortunately no variety of bread wheat has been found resistant to all of the physiologic forms of leaf rust, although several were resistant to eight of the twelve forms.

With the exception of Vernal emmer, S. D. 293, no other variety has been found resistant in the seedling stage to all the physiologic forms of leaf rust of wheat in these studies. On the other hand, no physiologic form of leaf rust has been discovered so far to which several varieties or strains of wheat have not been found resistant. In view of the results obtained by Hayes and Aamodt (10), Harrington and Aamodt (9), and Puttick (21), in connection with breeding wheat for stem rust resistance, it seemed very probable that wheats can be made still more generally resistant to leaf rust by selecting as parents of crosses those varieties which will combine resistance to the largest number of physiologic forms of leaf rust. Studies now being conducted with such crosses indicate that resistance to leaf rust may be combined in a similar manner. It should be emphasized, however, that a number of varieties, Kanred among the common bread wheats and especially certain varieties of the durums, emmers, and einkorns have gen-

erally exhibited a remarkably high resistance in field sowings. Most of these, however, under greenhouse conditions have not shown a reaction to all the physiologic forms at all comparable to their reaction under field conditions. Although many of the durums, emmers, and einkorns can be used as differential varieties, their susceptibility to a number of the forms is not so clear as is that of those varieties which have been selected and used for that purpose.

It is evident that what we in general call resistance to leaf rust of wheat is not due to one factor, but is caused by a number of different factors. Thus the high resistance shown by Malakoff, C. I. 4898, Turkey 47, and Norka, C. I. 4377 to six of the twelve physiologic forms of leaf rust, namely, 1, 2, 3, 4, 11 and 12, to all appearances is very similar to that shown by Mediterranean, C. I. 3332. The latter, however, is susceptible to physiologic forms 2, 3, 4 and 12, but resistant to 9 and 10, to which the first three varieties are susceptible. Other varieties show still other manifestations of this condition. Again, while some varieties like Malakoff possess a resistance which apparently is not easily changed, others, like Kanred and a number of durum and emmer varieties, may show a much higher resistance in the field than they do when grown in the greenhouse.

The resistance found in the strains of wheat, Malakoff, C. I. 4898, Turkey 47, and Norka, C. I. 4377 presents an interesting situation. These strains have all behaved very similarly, indicating that their rust resistance is likely to be due to the same factor or factors. The first two are considered by Clark, Martin, and Ball (3) as synonymous with Turkey, a white chaffed winter wheat. Norka is a brown chaffed spring wheat. While agreeing with Norka in reaction to leaf rust, Malakoff, C. I. 4898, and Turkey 47 differ markedly from a number of other lines of Turkey. In a similar way, Mediterranean, C. I. 3332, differs in its reaction to leaf rust from a number of other strains of Mediterranean wheat.

As the various strains of Turkey are grouped together in that variety largely because they possess in common such morphologic characters as winter habit, a white weak stem, awned spike, white glabrous glumes and hard red kernels, the lack of uniformity for rust resistance among such strains would indicate that the leaf-rust resistance of Malakoff, C. I. 4898, is not likely to be linked with any of these characters. For the same reason, the leaf-rust resistance of Mediterranean, C. I. 3332, is not likely to be linked with such characters as winter habit, purple stem, glabrous brown glumes, bearded spike, and soft red kernels.

From the above, it evidently is unsafe to draw general conclusions concerning agronomic varieties of wheat from the results obtained by the study of a single strain of the variety. If comparable results are desired in the

study of physiologic forms of leaf rust, the same pure-line resistant strain of the variety must be used by the various investigators. As resistance in most instances apparently is a physiologic character, or a combination of several such characters, rust-resistant lines of wheat can be distinguished with certainty only through their reaction to the rust. The rust and host are interacting organisms, and their reactions serve to characterize each other.

The twelve physiologic forms of leaf rust have been studied hardly long enough to justify any definite conclusion as to their fixity. The accumulated evidence, however, indicates that there is no very great change in their ability to infect varieties beyond a slight fluctuation, due probably to the effect of changing environmental conditions on the host.

The physiologic forms of the leaf rust of wheat apparently are very seldom found pure in any locality, but usually several are found mixed together. This is not surprising, as the leaf rust organism is a wind-blown pathogene. Wheat is grown quite generally throughout the eastern and central United States, and leaf rust is very common in this territory every year. The studies of Stakman et al (22) on the spore content of the upper air show that leaf rust urediniospores are found up to an altitude of 10,000 feet or higher. This indicates that spores may be blown long distances from their point of origin. When we consider that wheat is usually quite generally infected with leaf rust, and that wheat is so commonly planted, in some localities almost in continuous areas, it is not surprising that there is more or less mixture of physiologic forms of rust.

It is possible, however, that different climatic conditions in various parts of the United States may impose some limitations on the prevalence of the different physiologic forms. It is conceivable that the physiologic forms may differ in respects other than in ability to infect certain varieties of wheat. They may differ in the range of temperatures through which their spores will germinate, in ability to develop in the host under various climatic conditions, and in ability to survive the winter in the host tissue. The studies of Hursh (12) and Peltier (20) with physiologic forms of the stem rust of wheat have shown that these differ somewhat in ability to germinate and infect under some of these conditions. The varieties of wheat grown in any given region also undoubtedly play some part. While these factors may not entirely prevent physiologic forms from developing, they may play a considerable part in determining the relative prevalence of the forms.

It is evident, however, that both the existence of physiologic forms, with their differences in ability to parasitize varieties, and the apparent fluctuations in their distribution complicate exceedingly the problem of breeding wheat varieties resistant to leaf rust. This situation makes desirable a complete knowledge of the physiologic specialization in the rust and a

knowledge of the susceptibility of wheat varieties to the various physiologic forms. It is desirable that, if possible, some clew be obtained concerning the origin of such forms, or at least, whether new forms are being originated frequently. This investigation therefore offers no hope of immediate, complete solution.

The results here recorded can be considered only as merely introducing the problem. This study can be considered only as a preliminary survey of the question of specialization in the leaf rust of wheat. Other varieties and strains of wheat will be discovered, possessing resistance to physiologic forms of leaf rust. Such additional differential varieties will make possible the identification of forms which can not now be distinguished. With Malakoff the only differential variety, only two forms of rust could be distinguished; by the use of C. I. 3756 in addition, four forms were recognized.

It is necessary, also, to recognize that some of the varieties which have been discarded because of their susceptibility to all the cultures used in these investigations may show resistance to physiologic forms which have not yet been isolated in a pure condition. For example, it is very likely that Malakoff would have been discarded if the cultures obtained in the beginning of this work had been restricted to the great-plains region where physiologic forms of the leaf rust to which Malakoff is susceptible apparently predominate. The method followed in these studies of using cultures of rust from as many sections as possible, and the study of varieties in nurseries in various localities, it is believed, reduce the possibility of overlooking resistant varieties.

It must also be recognized that the information on susceptibility has been obtained on seedlings of the various varieties grown in the greenhouse. It does not follow that the reaction in the field will be identical. In fact, as stated, varieties which have shown promising resistance in the field have been more or less susceptible in the greenhouse studies. Such varieties are not being discarded but are being studied in the nurseries, the reason for the apparent difference in behavior being an important question for future investigation. While it is by no means definitely established, from the information at hand, it would appear that varieties resistant in the seedling stage in the greenhouse are likely to be fully as resistant or even more resistant to the same physiologic form in the later stages of development of the wheat plant in the field.

NOMENCLATURE OF LEAF RUSTS

It may be well to discuss here briefly the nomenclature which has been used in connection with the leaf rust of wheat. While the whole question of nomenclature of physiologic forms is too unsettled to attempt to draw final conclusions in regard to the name which should be used for this rust, yet a brief discussion of the situation with respect to this particular rust

and a few comparisons seem desirable. When Eriksson and Henning (7) separated *Puccinia glumarum* from the leaf rusts of the cereals and wild grasses, they proposed the name *Puccinia dispersa* for the remainder of the leaf rusts on wheat, rye, and wild grasses. Eriksson (5), as the result of further studies, recognized four specialized forms or varieties in this species: 1. *secalis* on *Secale cereale* with aecia on *Anchusa* spp.; 2. *tritici* on *Triticum vulgare;* 3. *bromi* on *Bromus arvensis* and *B. brizaeformis;* and 4. *agropyri* on *Agropyron repens,* the last three with aecia unknown at that time.

As the result of still further study, Eriksson (6) found that each of these forms was very closely specialized to a few hosts and considered that they were therefore worthy of being considered as species. He retained the name *Puccinia dispersa* for the form on rye. The leaf rust of wheat was named *Puccinia triticina;* that on species of Bromus, *Puccinia bromina;* that on *Agropyron repens, Puccinia agropyrina;* that on *Holcus lanatus* and *H. mollis, Puccinia holcina;* and that on *Trisetum flavescens, Puccinia triseti.* It is to be noted that these rusts are to be distinguished largely by physiological differences, host specialization, time of spore germination, etc., as only minor morphologic differences occur.

Since these studies of Eriksson were published, a number of investigators have extended our knowledge of the host specialization of this group and have demonstrated the aecial connection of several additional forms with different species of the families Boraginaceae, Hydrophyllaceae, Ranunculaceae, and Balsaminaceae. With one or two exceptions, there is a more or less close specialization to a few species of hosts, while the morphology is very similar. A number of investigators, following Eriksson's lead, have considered these specialized forms of rust worthy of specific rank and have given names to them. The writers (13) have discussed a number of those forms which have aecia on species of *Thalictrum,* but a fairly large number of other genera of the Ranunculaceae, as well as Boraginaceae, Hydrophyllaceae, and Balsaminaceae are known to include aecial hosts for the grass rusts of this group.

Some investigators, however, have not considered all of these forms worthy of specific rank. Thus Arthur and Fromme (1) list a number of such forms, including the leaf rust of wheat, having aecia upon different species of the family Ranunculaceae, as belonging to one species, *Dicaeoma clematidis* (DC.) Arth. Cunningham (4), concurring in this view but objecting to this name on the basis that it was a name first applied to the aecia and not tenable according to the International Rules of Nomenclature, considers that the name *Puccinia elymi* Westnd. should be used. Forms having aecia on species of the families Boraginaceae (*Puccinia dispersa, P. bromina*), Hydrophyllaceae (*Puccinia apocrypta* Ellis & Tracy), and Balsaminaceae (*Puccinia impatientis* (Schw.) Arth.), however, do not differ

morphologically from those with aecia on species of the Ranunculaceae more than the latter differ among themselves. In consequence, if species are to be founded entirely or largely on a morphological basis, it would seem that these also should be included. We would then have the interesting situation that the species as thus conceived would be at least very similar to Eriksson's original conception of the species *Puccinia dispersa.* However, it would be more desirable to use the name *Puccinia rubigo-vera* (DC.) Wint. for this species as thus conceived, because the name *Puccinia dispersa* has become established in the literature as applying only to the race of leaf rust found on rye. If aecial names are used, the name would be *Puccinia asperifolii* (Pers.) Wettst. There would be much less confusion involved in using the name *Puccinia rubigo-vera,* which was more or less extensively used for this group of rusts until superseded by the names proposed by Eriksson for the different races.

If the leaf rusts of rye, wheat, and wild grasses, with the general morphology of the leaf rust of wheat, are considered as one species, the situation would be more nearly comparable to that of the stem rust of wheat, *Puccinia graminis,* especially if the stem rust of timothy, with its aecia unknown, is considered as belonging to the latter species. The leaf rust species would then consist of a large number of races which, however, would be distinguished not only by the grass host to which they are specialized, but unlike *Puccinia graminis,*[7] also by the aecial hosts to which they are specialized. Upon this basis the race of leaf rust of rye would then be *Puccinia rubigo-vera dispersa* (or *secalis*) ; that of *Agropyron repens, Puccinia rubigo-vera agropyrina;* that of wheat, *Puccinia rubigo-vera triticina* (or *tritici*), with suitable names for other grass races. With this concept, the physiologic forms of the leaf rust of wheat would be denoted in a way similar to that employed for the physiologic forms of stem rust. Thus form 1 would be *Puccinia rubigo-vera triticina* p. f. 1, and the others would be designated similarly.

The writers believe that such a treatment of these leaf rusts is desirable, as it brings out most clearly the basis upon which they are distinguished. However, it is somewhat doubtful whether, because of the general use in the literature of such names as *Puccinia dispersa* for the leaf rust of rye, and *Puccinia triticina* for the leaf rust of wheat, such a change might not be more confusing than clarifying. It is largely for this reason that the name *Puccinia triticina* has been used in this paper. It should be kept in mind, however, that, if binomials are retained for these rusts, they apply to groups of rusts which can be distinguished from groups of similar morphology very largely by their physiologic specialization to certain hosts,

[7] The stem rust of timothy with its aecial host unknown but not producing aecia on *Berberis vulgaris,* the aecial host for the rest of the species, may be an exception.

and therefore are comparable to races or varieties in such species as *Puccinia graminis.*

SUMMARY

1. The leaf rust of wheat, *Puccinia triticina,* consists of at least twelve physiologic forms distinguished by the manner in which they infect eleven selected differential strains of wheat.

2. Twenty-five strains of wheat have been found to be more or less resistant to one or more of these forms. These are Malakoff, C. I. 4898; Turkey 47; Norka, C. I. 4377; Mediterranean, C. I. 3332; Mediterranean, C. I. 5776; Mediterranean (Imperial Amber), C. I. 4860; Hussar, C. I. 4843; Beloglina, C. I. 1544; Beloglina, C. I. 2239; Pacific Bluestem, C. I. 4821; Webster, C. I. 3780; unnamed spring wheats, C. I. Nos. 3756, 3778, 3747, and 3779; Peliss, C. I. 1584; Acme, S. D. 284; Arnautka, Minn. 337; Monad, C. I. 3330; Mindum, C. I. 5296; Khapli emmer, C. I. 4013; Vernal emmer, S. D. 293; Double einkorn; and Einkorn, C. I. 2433.

3. Of these, eleven varieties, comprising eight differential groups, have been found to distinguish best the twelve physiologic forms of the rust. These are Malakoff, C. I. 4898; Turkey 47; Norka, C. I. 4377; Mediterranean, C. I. 3332; Hussar, C. I. 4843; Democrat, C. I. 3384; Webster, C. I. 3780; and the unnamed spring varieties, C. I. Nos. 3756, 3778, 3747, and 3779.

4. The physiologic forms of the rust are distinguished by the reaction shown by these varieties when infected with leaf rust. No form has been found so far to which all of these varieties are susceptible. On the other hand, other than the variety Vernal emmer, S. D. 293, no variety has been found which in the seedling stage in the greenhouse has proved highly resistant to all twelve of these forms.

5. The physiologic forms of the leaf rust of wheat are not generally found pure in the field, but practically always several are found mixed together.

6. In order to study and identify such forms, it is necessary to obtain them in a pure condition. For this purpose, pure lines of the rust have been developed by starting each culture from a single urediniospore.

7. Physiologic forms of the rust are neither fixed nor limited in their distribution. Apparently those forms to which Malakoff, C. I. 4898, is susceptible are the most prevalent in the central west, while those to which this variety is resistant are more common in the east and south.

8. Agronomic varieties of wheat are not necessarily uniform in their reaction to leaf rust. Different strains of such varieties may differ markedly in their reaction.

PURDUE UNIVERSITY
AGRICULTURAL EXPERIMENT STATION,
LAFAYETTE, INDIANA.

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PLATE VI

Types of reaction shown by wheat to leaf rust, *Puccinia triticina*. ×3.

0. Highly resistant; no uredinia formed; flecked.

1. Very resistant; more or less flecked, accompanied by few small uredinia.

2. Moderately resistant; uredinia fairly abundant and of moderate size, accompanied by necrosis.

3. Moderately susceptible; uredinia fairly abundant and of moderate size, sometimes with chlorosis.

4. Very susceptible; uredinia abundant, large, sometimes in more or less pronounced green islands.

Upper row from left to right: (0) Norka by form 12; (0) Malakoff by form 1; (1) Malakoff by form 3; (1) C. I. 3780 by form 3; (2) Peliss by form 9; (2) C. I. 3756 by form 9.

Lower row from left to right: (3) Arnautka, Minn. 337 by form 5; (3) Imperial Amber by form 5; (4) C. I. 3769 by form 9; (4) C. I. 3756 by form 12 (compare under 2); (4) Norka by form 9 (compare under 0); C. I. 3747 by form 9.

PLATE VII

Comparison of four differential varieties of wheat for reaction to three physiologic forms of leaf rust, *Puccinia triticina*.

	1. Form 3	2. Form 9	3. Form 11
A. Norka, C. I. 4377	resistant	susceptible	resistant
B. Webster, C. I. 3780	resistant	susceptible	moderately resistant
C. Malakoff, C. I. 4898	resistant	susceptible	resistant
D. Democrat, C. I. 3384	susceptible	resistant	resistant

Note the close agreement of Norka (A) and Malakoff (C).

PLATE VIII

Comparison of four differential varieties of wheat for reaction to three physiologic forms of leaf rust, *Puccinia triticina*.

	1. Form 3	2. Form 9	3. Form 11
A. C. I. 3779	moderately susceptible	susceptible	susceptible
B. Mediterranean, C. I. 3332	moderately susceptible	resistant	resistant
C. Hussar, C. I. 4843	resistant	moderately resistant	resistant
D. C. I. 3778	moderately resistant	moderately resistant	susceptible

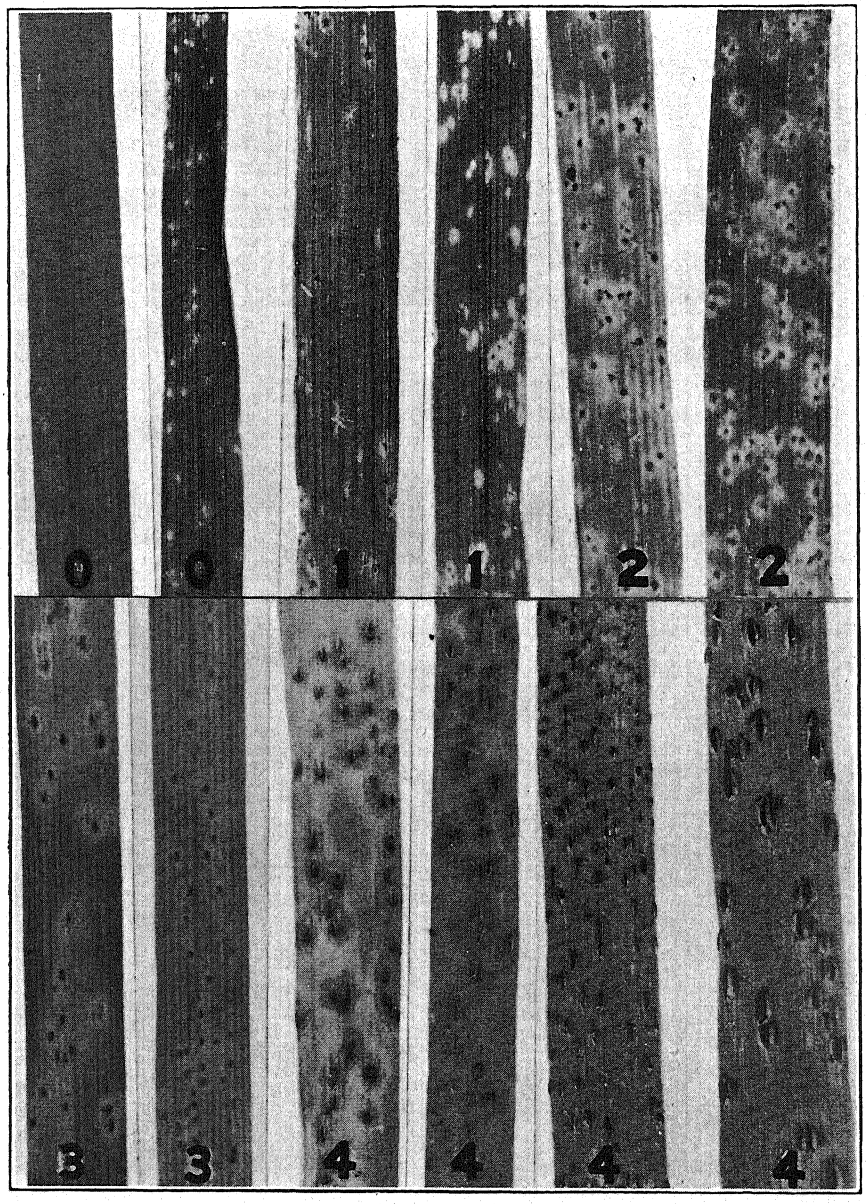
PLATE IX

Difference in reaction to the same physiologic form, I, of leaf rust, *Puccinia triticina*, shown by different strains of the same variety.

A. Variety Mediterranean; Resistant, (1) strain C. I. 3332, (2) strain C. I. 5776; Susceptible, (3) strain C. I. 5834, (4) strain C. I. 1395, (5) strain C. I. 5614.

B. Variety Turkey; Resistant, (2) Malakoff, C. I. 4898, (3) Turkey, strain 47; Susceptible, (4) Malakoff, Minn. 1479, (5) Turkey strain Minn. 1548, (6) Turkey strain Minn. 1549, (7) Kharkoff, C. I. 4897.

B1. Norka, C. I. 4377, agreeing in resistance with the Malakoff, C. I. 4898, and Turkey 47, strains of Turkey.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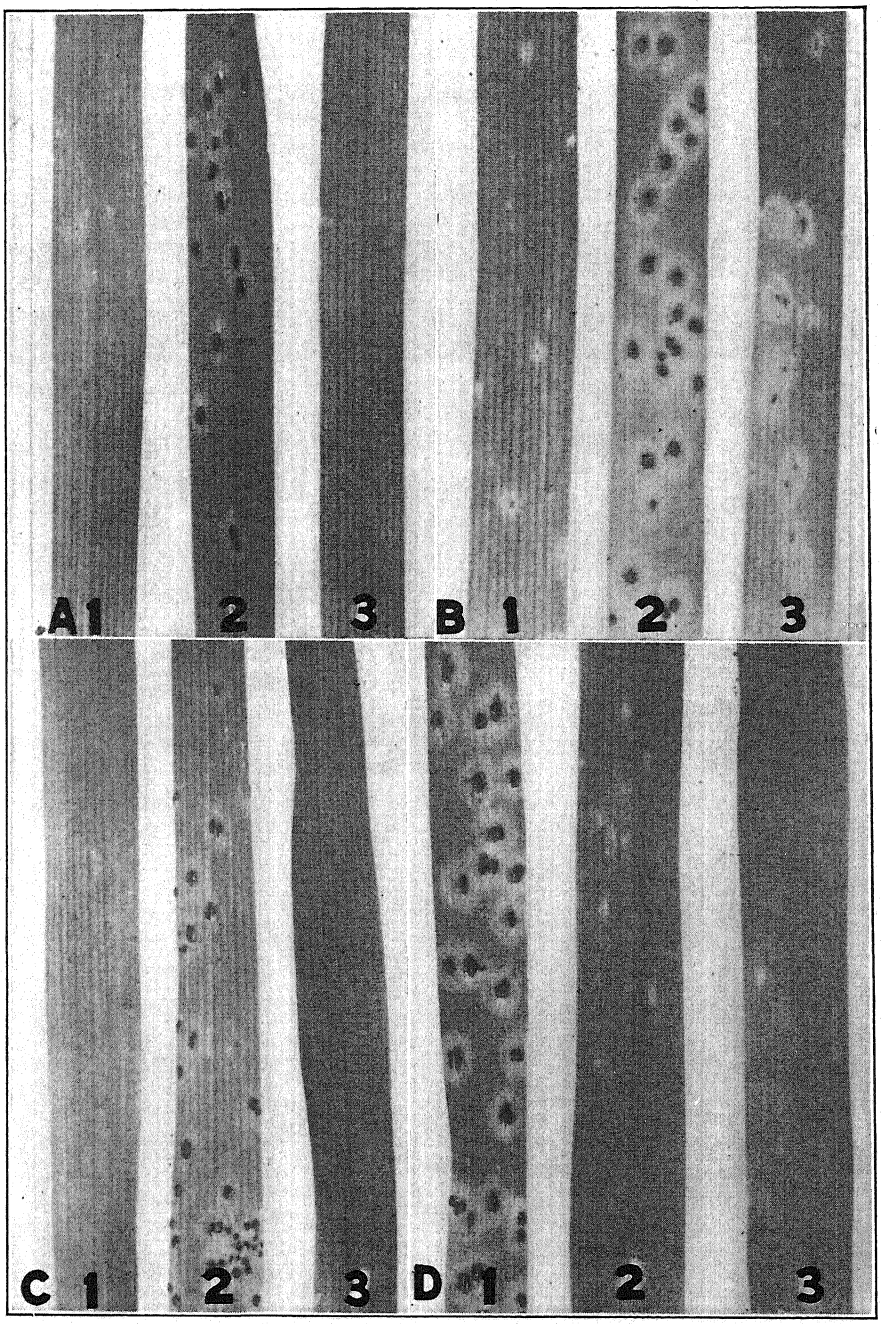
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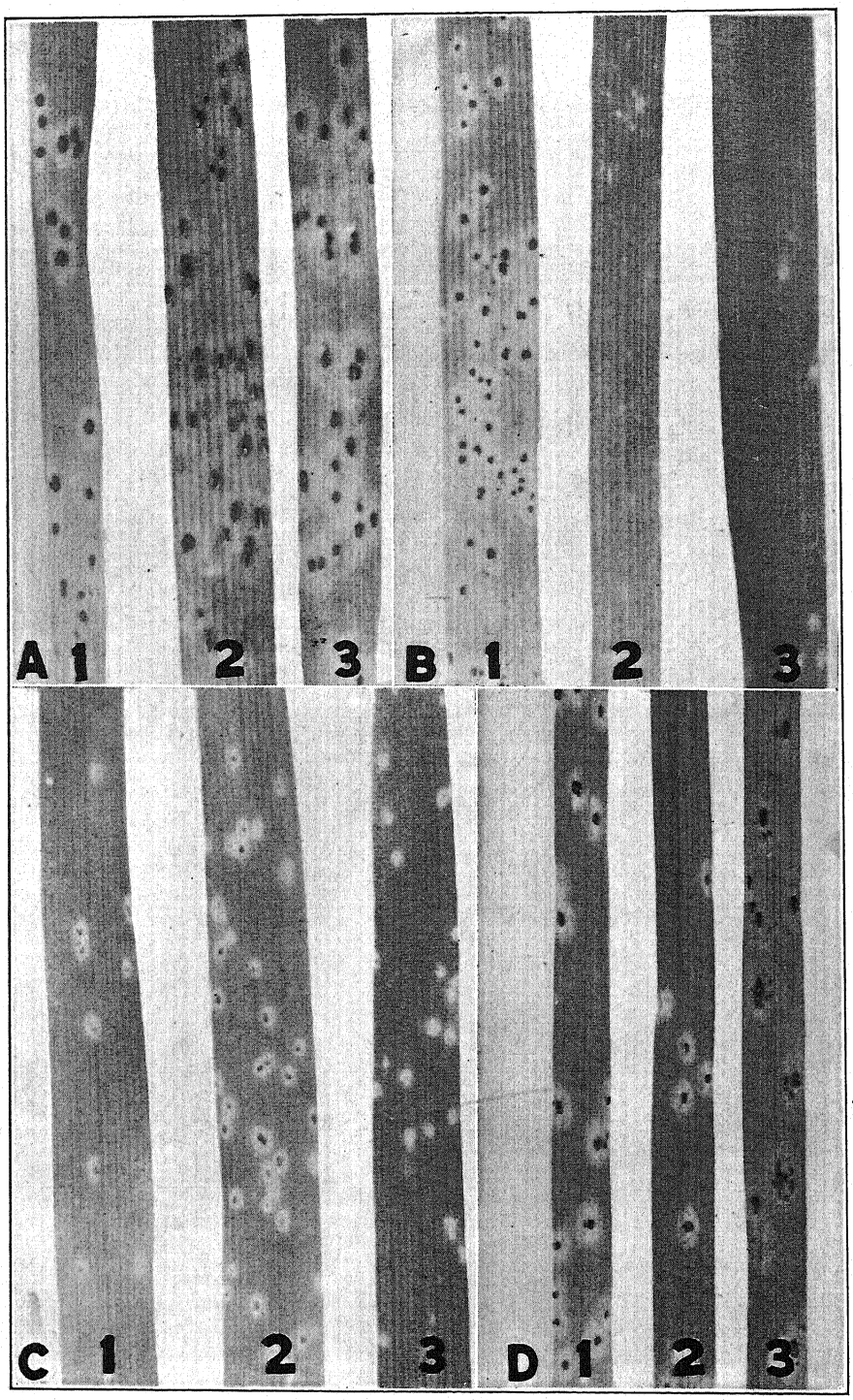
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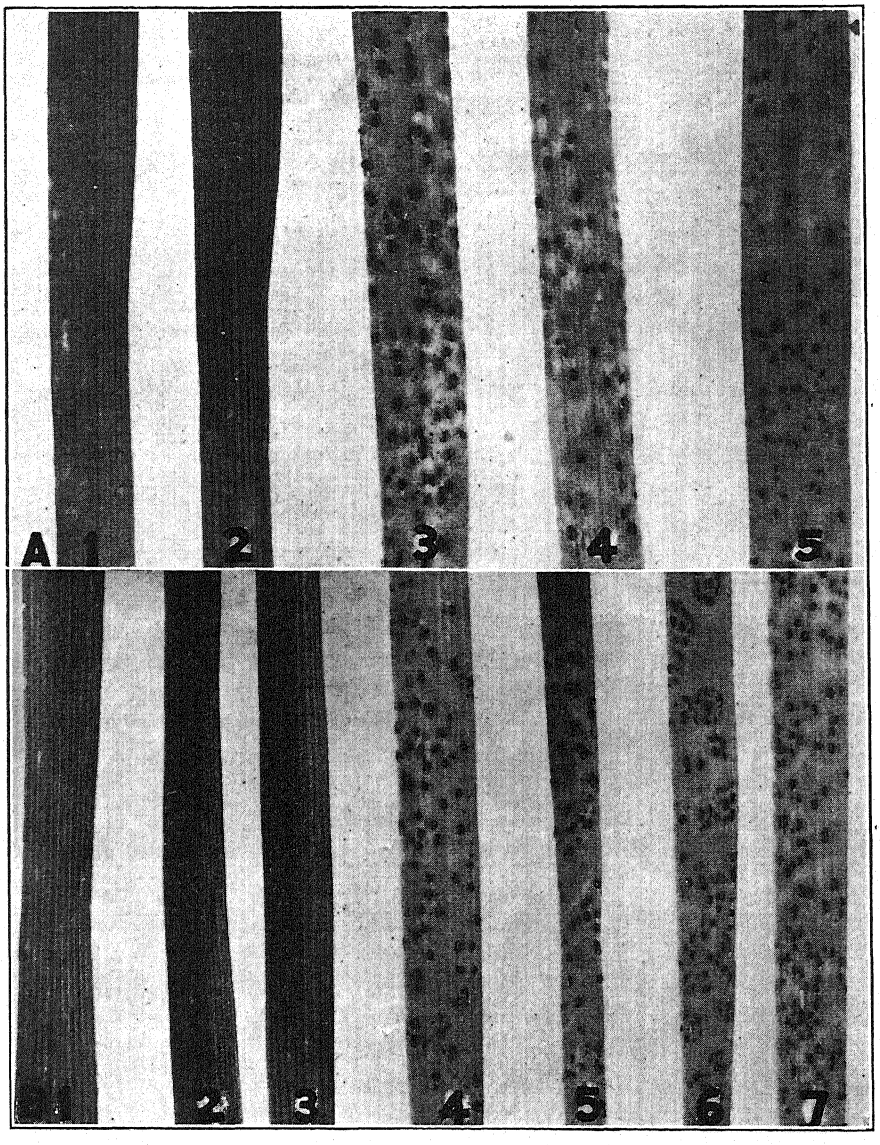
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THE OVERWINTERING OF PHYTOPHTHORA INFESTANS (MONT.) DE BY.

HELENA L. G. DE BRUYN

WITH THREE FIGURES IN THE TEXT

INTRODUCTION

The overwintering of *Phytophthora infestans,* the potato blight fungus, is still an unsolved problem. Since De Bary's idea (2, 3, 4) that the surviving mycelium in the diseased potato tuber is the only mode of hibernation of the fungus, numerous efforts have been made to settle this question experimentally, but no definite, convincing results have been obtained. Many observed facts still point to the inadmissibility of De Bary's opinion, and suggest other explanations for the overwintering of the fungus. The most satisfactory one is that of the hibernation of the fungus in the field; and as long as no striking results are obtained to show that this is impossible, it may be looked upon as explaining the observed facts far better than the theory that the diseased tuber is the only way by which the fungus remains alive. This does not mean, however, that mycelium hibernating in the tuber may never be the source of infection of the foliage of the succeeding year's crop. It is quite possible that under special circumstances the diseased tuber may be, in some way or other, the origin of the outbreak of the disease. All outbreaks, however, cannot be explained in this way, *e.g.,* those starting very late in the season during dry summers. The first thing to esablish is whether overwintering in the field can occur, and then to determine what part such overwintering plays in the life history of the fungus.

The problem may be solved by field observations and by experimental methods. The field method presents enormous difficulties, although Brooks (6) and his co-workers gathered much evidence for the persistence of *Phytophthora* in the field during their investigations. However, the study of the development of the fungus under different conditions in the laboratory is also of much value. In this respect the discovery of the occurrence of oospores in pure cultures by Clinton (11) and by Pethybridge and Murphy (26) has been of great importance, since the failure to find resting spores was one of the principles underlying De Bary's opinion. The discovery of an oospore in pure culture on sterilized soil (8) increased the probability of the occurrence of oospores under natural conditions.

The saprophytic life of the fungus is closely related to the problem of hibernation. The author has proved that *P. infestans* is not so strict a parasite as was thought formerly. It was also ascertained that it can live in pure cultures on different types of soil (7). The existence of the fungus in the soil, and its growth there in the absence of living potatoes, strengthens the idea of hibernation independent of the potato tuber.

The great problem concerning overwintering which remains to be solved, however, is the question whether the fungus can endure the low temperatures of winter. No definite results were obtained on this matter in previous work. Following this, the question arises as to the form in which the fungus can withstand the cold. Are oospores necessary, and under what conditions are they formed? The present investigation was undertaken to settle these questions, since more exact methods were now possible in low temperature experiments, owing to the availability of a refrigerator. At the same time, additional information was obtained concerning the saprophytic life of the fungus.

THE SAPROPHYTISM OF PHYTOPHTHORA INFESTANS

About the beginning of the present century, various investigators succeeded in growing the blight fungus in pure culture on different media, thus proving that it is not so strict a parasite as was thought formerly. Most of the investigators considered its saprophytism to be rather narrow. Enumerating the various media used, however, makes it evident that a great variety of substances are able to nourish the fungus. *P. infestans* may require special conditions for its growth, but is not restricted to a few substances for food material.

Good growth was observed on many plant media, viz., potato, oat, maize, lima and white bean, Spanish melon, and vegetable marrow [Matruchot and Molliard (23), Brefeld (5), Clinton (9) (10) (11), Jones, Giddings, and Lutman (13), Pethybridge and Murphy (26)]. These substances were prepared in different ways and used alone, moist, or mixed with agar or gelatin. The potato, Spanish melon, and vegetable marrow were also used in living condition.

Hecke (12) used decoctions of plums, tomatoes, cherries, and potato leaves in moist chambers and in watch glasses for the study of the germination of the conidia. He writes: "By transferring these several times one gets at last a pure culture." From this it is not clear whether Hecke really grew the fungus in pure culture on these media for any length of time. Attempts by the present author to grow the fungus in plum and cherry decoctions of different concentration or neutralized with KOH gave unsatisfactory results. Plum and cherry decoctions, therefore, are not being added to the list of media on which *P. infestans* can thrive.

In addition to those made from vegetable material, synthetic media were used. Matruchot and Molliard (23) state that the fungus grew in a three per cent solution of glucose in water. Jones, Giddings, and Lutman (13) describe good or fair growth on some ten synthetic media of varying composition, while Kossowiesz (14, 15, 16, 17, 18, 19, 20, 21) used about 50 different combinations successfully. Kossowiesz proved that *P. infestans* can utilize uric acid, hippuric acid, glycocol, or guanin as sole sources of carbon and nitrogen; that urea, guanidin, potassium sulphocyanide, nitrite, nitrate, or calcium cyanamide can be the sole sources of nitrogen; and that as the sole carbon compound in the solution, either saccharose, dextrose, or mannite suffices. *P. infestans* therefore does not require special substances as nutriment, but can obtain the necessary carbon and nitrogen in quite different forms.

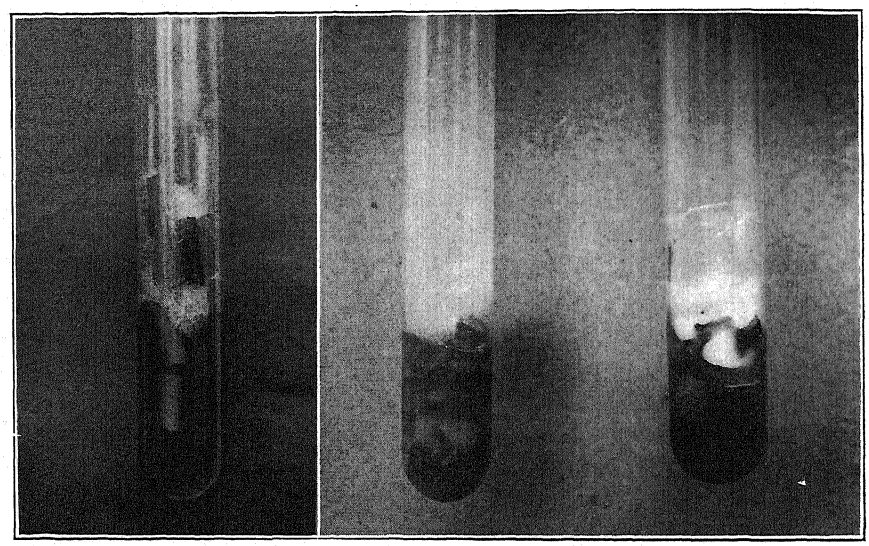
Kossowiesz used liquid media. Most of the earlier investigators were of the opinion that it made no difference whether liquid broth or solidified agar or gelatin were used. Clinton preferred agar to gelatin. Löhnis (22) stated that she obtained better results when one instead of two per cent agar was used. According to the writer's experience, *P. infestans* requires rather moist media. Although agar or gelatin may give good growth, the fungus grows better on a mixture of firm material with liquid. For the experiments described here the standard medium consisted of three or four maize kernels in an excess of water. A thick mat was formed on the surface of the liquid, and pieces of this mat were very handy for transferring.

The growth of *P. infestans* in pure culture on different types of soil (7) also is of importance in relation to its saprophytic life. Bog soil, clay, and leafmold proved to be better media than sand, although on the last named slight growth occurred. Best of all is sterilized manure, which could be added to the list of media in 1923 (8). The fungus has remained alive on bog soil with no other food material for nearly four years, and has been transferred only when the soil in the tube became dry. On manure the fungus has now been living for two and one-half years. The constituents of the soil, as well as those of manure, suffice as nourishment for the fungus.

Since the organic parts of the soil form probably the chief substances on which the mycelium feeds, and since these consist partly of old plant remains, attempts were made to determine to what extent portions of different plants could be used as media for growth. The condition of the plant before sterilization was observed to influence the behavior of the fungus. Parts taken from living plants gave different results from those obtained when dead and discolored parts of the same kind of plant were used. The

plants were cut into pieces, placed in tubes and covered with distilled water. Enough water was used so that after sterilization only the upper portions were above the water level. The fungus was transferred to these tubes from the stock culture on maize kernels.

On the best media, growth was noticeable within a few days after inoculation. On certain others no growth could be recognized at the beginning, although it started later and resulted, in the end, in very good mycelial development. It made no difference whether the tubes were used immediately or some months after sterilization. On certain media only



a b

FIG. 1. a. Culture of *Phytophthora infestans* on wheat straw.
b. Culture of *Phytophthora infestans* on fresh and dead stems of the same variety of potato, a fortnight after inoculation.

slight growth took place at the beginning and practically no increase of mycelium occurred during six months. In some of these cases of very dubious growth, the original piece of mycelium was transferred after four and one-half months to a more suitable medium. Excellent development resulted, thus proving the inhibitory action of the previous medium on the growth of the fungus, and the strong vitality of the organism. There also is a difference in the proportion of tubes in which the fungus developed. On the good media, growth was obtained in all the tubes inoculated, while in other cases growth was observed in only a very small number of tubes.

Table 1 gives a list of the plants used and the results obtained (Figs. 1 and 2).

TABLE 1.—*Growth of Phytophthora infestans on various sterilized vegetable media*

Medium	Character of growth
Oat straw	Very good
Wheat straw	do
Rye straw	do
Rye haulms; fresh	Good in 50% of the tubes
Grass; fresh	Fair
Potato stem; dry, brown	Very good
do fresh, green	Slight after one month, good after two months
Equisetum; dry, brown	Very good
do fresh, green	Very slight
Bean stem; dry, brown	Good
do fresh, green	Slight after one month, fair after two months
Horse bean stem; dry, brown	Good
do fresh, green	Very slight after two months in two tubes out of eight
do pod; dry, black	Good
do pod; fresh, green	Slight after one month, good after two months
Rhubarb flower stalk; dry, brown	Good
do petiole; fresh, green	None
Chrysanthemum leucanthemum; dry, brown	Good
do fresh, green	Very slight after three months in one tube out of six
Beet root; fresh	None
do petiole; dead	do
do do fresh	do
do flower stalk; dry from previous year	Good
do fresh	None
Tomato fruit;	None
do stem; dry	Very good
do fresh	Good after three months in one tube out of seven
Carrot root	Fair
Pteridium aquilinum; dry, brown	Good
Calluna vulgaris; dry, brown	Slight in one tube out of six
Larix needles; dry, brown	None
Mixture of various plants gathered at the side of a ditch; dry, brown	Good

The most striking fact is the far better growth of the fungus on sterilized, old, dried, brown parts of plants than on sterilized fresh plant parts. In some cases the fungus flourished on the sterilized, withered plant re-

mains, while no growth whatever took place on the same kind of plant sterilized in fresh condition. When growth occurred on fresh plant parts, it was always much less than that which occurred when the fungus was grown on dead parts. It is evident that the fungus prefers partially decayed plants, and this leads to the conclusion that, on the whole, *P. infestans* possesses more saprophytic than parasitic tendencies.

The explanation of this fact must be either that the substances in the plants are altered to such an extent through withering that only after this can they be utilized as food by the fungus, or that the fresh plants contain substances which inhibit growth. Since *P. infestans* can grow in solutions of widely varying and rather simple composition, it is not very likely that food material is lacking in any plant decoction. It is highly probable, therefore, that the second supposition holds true.

The inhibiting factor may be either some specific substance or the concentration in which one or more of the substances are present. Jones, Giddings, and Lutman (13), as a result of their experiments with synthetic media, are of the opinion that a high concentration of a solution prevents growth. Löhnis (22) disproved this by cultivating *P. infestans* on bean agar with 60 per cent of sugar. Although high osmotic pressure of itself may have no inhibiting effect, it may be true that certain substances become toxic if present above a certain concentration. The fact that in some cases development occurred in only a very small number of tubes may point in this direction, as also may the slight growth on some media. However, in those cases where good growth started after one or two months the explanation is rather difficult.

In connection with the study of the resistance of the potato to blight, it is important to know what factor really inhibits growth.

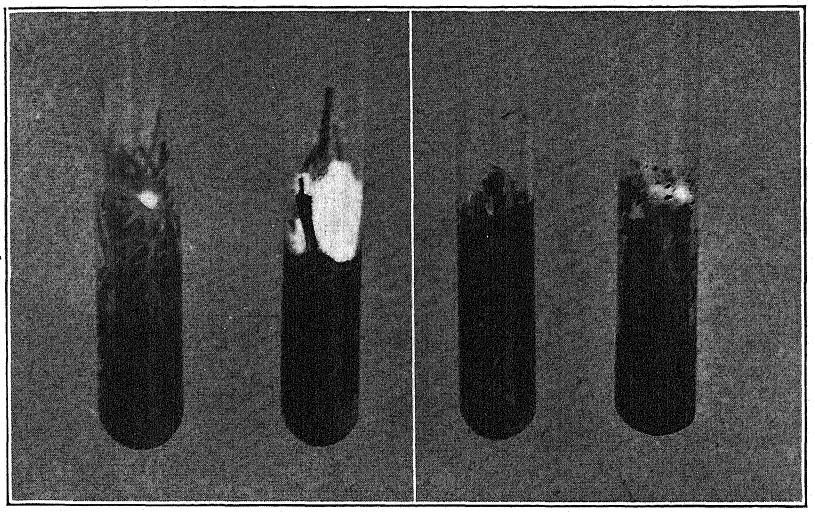
The number of plants which can serve as nutriment is large, as can be seen from table 1, and they do not belong to closely related families. The plants used were, on the whole, only those which happened to be at hand in both conditions, brown and dead, and fresh and green, respectively.

The experiment shows that a saprophytic life of *P. infestans* is quite possible in the field, independent of the potato plant. In all types of soil some of the plant remains mentioned will be found in all stages of decay, especially the straw of cereals and grasses. The quantity of suitable organic constituents may vary according to the type of soil and treatment of the field, but some of them will be present in all soils.

In admitting the possibility of the fungus living in the soil, no difference is assumed in the behavior of the fungus towards soil in a sterilized state and under natural conditions. Competition with other organisms might check growth in unsterilized soil, but whether *P. infestans* would

lose or win in this struggle for existence is unknown. In the author's experiments the fungus was found on the whole to possess strong vitality.

Murphy and McKay (25) object to the idea of the fungus being able to live in the soil for long periods. Murphy (24) had carried on experiments with unsterilized soil, and came to the conclusion that soil contaminated with conidia of *P. infestans* retains its power of infection for forty-four days. According to him, it is the formation of germ tubes and secondary conidia which prolongs the vitality of the spores. He does not accept the idea of a growth of the fungus through feeding on the soil contents. His results, however, can be explained as easily by a saprophytic life of the fungus in the soil as by the prolongation of its existence by the formation of secondary conidia. It may have been due to unfavorable conditions that, in Murphy's experiments, growth stopped after forty-four days. Finely sifted potting



a b

FIG. 2. a. Culture of *Phytophthora infestans* on fresh and dead *Equisetum*, a fortnight after inoculation.
b. Culture of *Phytophthora infestans* on fresh and dead stems of *Chrysanthemum leucanthemum*, a fortnight after inoculation.

loam and silver sand were used. Both probably contained little organic matter, and therefore but little nourishment. In the present author's opinion the results of Murphy's experiments are not in contradiction with the idea of a saprophytic life of *P. infestans* in the soil.

THE OCCURRENCE OF OOSPORES AND RESTING FORMS

The oospores of *P. infestans* are known only from pure cultures. They were observed on oat-juice agar, Clinton (11), ground Quaker oats agar, Pethybridge and Murphy (26), cornmeal agar and oat-extract agar, de Bruyn (8). Abnormal structures called resting spores were described by Jones, Giddings, and Lutman (13) and by Löhnis (22).

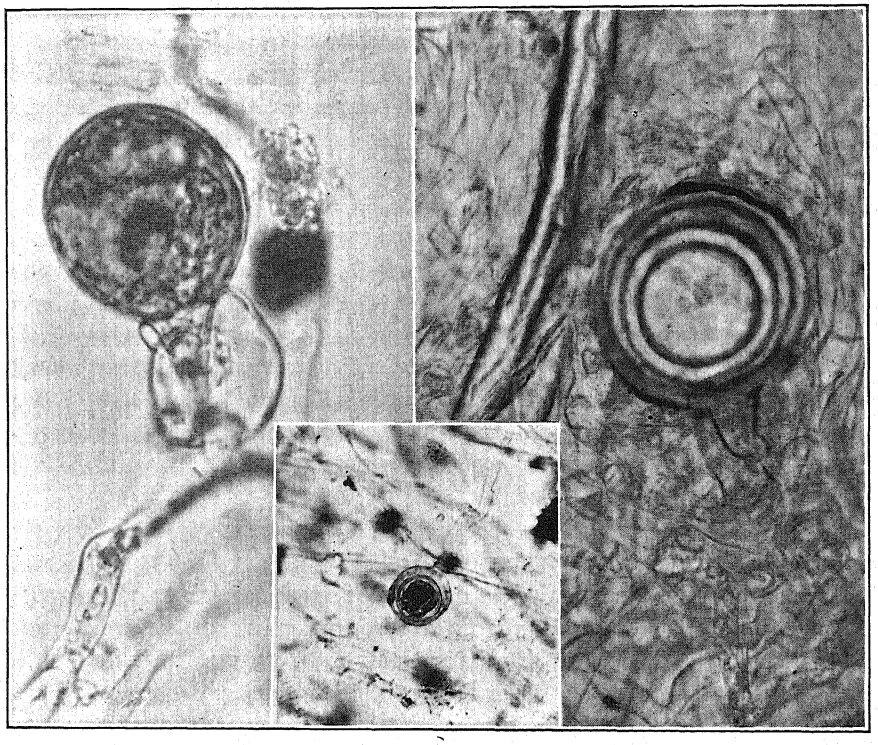
The presence of oospores on the new media used in the present experiments was often very difficult to demonstrate, chiefly when they occurred only in small numbers. The portion of plants used, especially the dead parts, contained all kinds of fungous spores before sterilization, thus making errors very easy. The same held true for the various types of soil. On bog soil (8), however, one oospore developed parthenogenetically and was discovered still attached to the mycelium. (See Fig. 3a.)

Definite oospores with antheridia attached were found in great abundance on the three kinds of cereal straw and on fresh rye haulms. Fig. 3b is an illustration of a young oogonium with an antheridium, from a culture on wheat straw, and shows distinctly that the antheridium is of the amphigynous type. Fig. 3c represents an older stage from a culture on rye straw, the oospore being already formed. The antheridium is also visible.

A tendency to form oogonia was observed on the medium consisting of dead *Equisetum* haulms. In only a few of these oogonia did a thick inner wall develop. Oospores were thus formed parthenogenetically. Very few antheridia were discovered. The bulk of the oogonia did not develop any further. They may be described as dark yellow, round bodies at the ends of hyphae. Their resemblance to true oogonia leads one to regard them as such. The oospore-like bodies mentioned by Rosenbaum (28) as occurring on oat-agar were probably similar. While these oogonia did not develop further, owing to the absence of antheridia, they were abundant in cultures on dead *Equisetum* haulms, and were observed in much smaller numbers on many other media. On dead *Pteridium aquilinum* they were easily found, but a thorough search had to be made on dead and on fresh potato stalks, on dry and on fresh bean stems, on dry *Chrysanthemum leucanthemum*, and on some other media, since only one, two or three were present. It is therefore of no importance to mention all the media on which the oogonia occurred so sparingly, since they may easily have been overlooked in other cases.

The media on which many true oospores were found (viz. the cereals), as well as those on which the fungus showed a tendency to form oogonia (*Equisetum* and *Pteridium aquilinum*), are all media composed of plants with a high percentage of silica. Clinton (11), after finding oospores on oat-juice agar, states that chemical analyses of oats show that they have

higher percentages of ash, fat, and lecithin than the other cereals. It seems that the higher per cent of ash is due largely to silica, and Clinton concludes: "So these constituents of the ash are apparently not the favorable factors." The above mentioned results, however, make it appear very likely that the percentage of silica present in the medium does influence the



b a c

FIG. 3. a. Parthenogenetic oospore from a culture on bog soil × 260.
b. Oogonium and antheridium from a culture on wheat straw × 900.
c. Oospore from a culture on rye straw × 700.

formation of oogonia in *P. infestans,* and probably also affects the production of antheridia and thus the formation of oospores. The quantity of silica, even in plants of the same kind grown under different conditions, will not be uniform. This may afford an explanation of the contradictory results often obtained by various investigators in connection with the formation of oospores on special media. Neither must it be forgotten that all cultures are grown in glass tubes.

In addition to oogonia and true oospores, all kinds of abnormalities are very frequent on the media mentioned and on others. The different types of resting spores described and figured in detail by Jones, Giddings, and Lutman (13) were often found. These resting spores are of very different forms: sometimes they are very difficult to distinguish from oogonia, while in other cases all stages are present, from round, yellow bodies to only slightly swollen or thickened mycelium. The swelling of the mycelium may be intercalary, and in this case the resemblance to a germinated spore is striking. One may as well call this a germinated oogonium as merely a swelling of the mycelium, and it would also be quite justifiable in such a case to speak of a chlamydospore and not of an oogonium. The thickened mycelium may also be strongly branched. Even conidia may have a thickened wall, often, though not always, of a yellow color. Many degrees of development of all these abnormalities exist. One might call them abnormal or not fully developed oogonia, conidia, or mycelium. In many cases the relation to the specific part of the fungus is clear; but, as has been said, in a great number also it is doubtful. It seems best to give them all one collective name, and for this the term "resting forms" is proposed, since this includes the abnormal mycelium as well as the spores. The function of these resting forms will always be the same, the thick wall and the presumed change in the constitution of the protoplasm suggesting adaptation to unfavorable circumstances.

If this name is accepted, the different organs which *P. infestans* may possess are the following: mycelium; conidia; oospores with a thick wall still surrounded by the oogonial wall, being developed either parthenogenetically in the absence of antheridia or sexually when antheridia are present; oogonia with a more or less thick wall; and resting forms, this term to include all those forms which differ from the others and which are characterized by the presence of a thick wall.

These resting forms are found in greater abundance on some media than on others; on certain media also some special form may occur more generally. Next to food, their presence will be dependent on external circumstances and will therefore not always be quite constant. However, there is no need to mention the media and the form on which the fungus occurs on them in more detail.

Since the formation of resting forms is a normal reaction of the fungus to unfavorable conditions, such as unsuitable food or drought, they probably will occur under the same circumstances in nature. It also is very likely that oospores are produced under natural conditions, although the problem of their occurrence in nature has not yet been solved. There is a better chance of finding them on old grass and cereal stubble than on old

potato plants. If oospores are found it remains to be proved that they are really those of *P. infestans*. Not until methods of causing the oospores to germinate have been discovered will it be worth while to make a thorough investigation.

THE BEHAVIOR OF PHYTOPHTHORA INFESTANS IN RELATION TO LOW TEMPERATURES

In 1922 the author (7) published results of experiments concerning the resistance of *P. infestans* to low temperature. Pure cultures of the fungus were exposed to normal winter weather conditions. In 1920–21 the fungus endured a temperature of $-9°$ C., but during the winter of 1921–22 a temperature of $-12°$ C. was reached and all the cultures died. The following experiments show that the fungus can withstand a temperature much lower than $-12°$ C.; and this suggests that the failure in 1921–22 must have been due to mistakes in technique. In the first place, the number of tubes used was too small, because the fungus survives in only a small percentage of cases. Secondly, the cultures were put out-of-doors too soon after transferring, since the fungus probably requires some little time for proper development before it can resist abnormal conditions.

In the following experiments a refrigerator was used; thus it was possible to regulate the temperature and the time during which the fungus was exposed to it. The lowest temperature which could be used was about $-25°$ C. Since nothing is known about the possible existence of physiological strains of *P. infestans,* the same strain—isolated in September, 1920, from diseased potato plants—was used throughout the experiments to prevent complications in the results.

In the first experiment the influence of the time of exposure, as well as of the treatment of the fungus after freezing, was studied. The whole experiment lasted for ten days, and every two days a certain number of tubes were removed from the refrigerator. Some of the cultures were put at once into an incubator at about 18° C., thus undergoing a sudden change of temperature of about 35° C. The rest were placed for twenty-four hours in a cool cellar at a temperature of $-2°$ C., next for twenty-four hours in an incubator at $+2°$ C., then for about ten days in a cellar at a temperature of 6° C., and finally they were transferred to new media and kept in an incubator at about 18° C. The fungus in the first lot of tubes had been transferred after thawing of the medium. The original cultures used in the experiment were also kept in the incubator. It was perceived that in some cases growth started after some weeks in the old tubes, while no development could be observed on the new media. New transfers were made with satisfactory results. In the later experiments, therefore, the cultures

were always kept for a month at the optimum temperature before transfers to new media were made. The fungus was thus able to renew growth and refill the tube. In the first experiment the temperature in the refrigerator was not very constant, varying from −9° C. to −17° C. On each of the first three days, however, −17° C. was reached. All the cultures were at least one and one-half months old, and the majority still older, before they were subjected to freezing. The results are given in table 2.

TABLE 2.—*The effect of keeping cultures of Phytophthora infestans for different lengths of time in different types of soil at a temperature of −9° C. to −17° C., and then thawing them gradually or suddenly*

Cooling period in days	Kind of soil: Bog soil		Sand		Leaf mold		Clay		Manure		Totals
	Change of temperature and number of cultures which survived										
	Sudden	Gradual	Sudden	Gradual	Sudden	Gradual	Sudden	Gradual	Sudden	Gradual	
2	2[a]/2	1/2	0/1	0/2	0/1	0/2	0/0	0/1	0/0	0/0	3/11
4	2/2	2/2	0/1	0/2	0/1	1/2	0/0	0/1	0/0	0/0	5/11
6	1/2	0/2	0/1	0/2	1/1	0/2	0/0	0/1	0/0	0/0	2/11
8	1/2	0/2	0/1	0/2	1/1	0/2	0/0	0/0	0/0	1/1	3/11
10	0/2	0/4	0/1	0/4	1/1	0/1	0/0	0/1	0/0	0/0	1/14
Totals	6/10	3/12	0/5	0/12	3/5	1/9		0/4		1/1	

[a] Numerator = number of cultures which survived; denominator = total number of cultures.

The fungus can endure a temperature of −17° C. The length of time during which it is exposed to this temperature is of no great influence since about the same percentage of survival was obtained with two, four, six, or eight days exposure. Only after ten days cooling was there a reduction in the number of cultures in which the fungus remained alive. The difference, however, is so slight that not much stress can be laid on this fact. Growth is somewhat better after a sudden change of temperature than after a gradual change. Richter (27) found, in his experiments with *Aspergillus niger*, that the survival of the fungus after freezing depended on the condi-

tions prevailing after thawing. At optimum temperature immediately after cooling, the fungus started to respire again and to form new mycelium; at a less favorable temperature, *Aspergillus* did not revive. *P. infestans* did not show this sharp difference, although, when placed at the optimum temperature immediately after cooling, it grew somewhat better than when brought to the optimum temperature gradually.

On the whole, the proportion of cultures that survived the low temperatures was rather small. A great difference was found in the behavior of the fungus when grown on various media. This points to the probability that not the mycelium, as in *Aspergillus niger*, but some special form of the fungus may be responsible for its survival, this special form being produced more readily on one medium than on another. This supposition would also explain the rather long time required in some cases for the renewal of growth, and also the irregular and low percentage of survival. Even cultures on the same medium are unequally resistant to cold, a point which is further demonstrated by the next experiment, in which the influence of different temperatures was studied.

TABLE 3.—*Effect of keeping cultures of Phytophthora infestans at different low temperatures for five days*

Medium	Temperature and number of cultures which survived		
	−12° to −15° C.	−17° to −20° C.	−21° to −25° C.
Clay	0[a]/10	0/10	0/10
Sand	0/10	0/10	0/10
Bog soil	2/10	0/10	2/10
Leaf mold	1/5	0/5	0/5
Manure	5/10	7/10	8/10
Maize kernels	0/10	0/10	0/10
Rye straw	1/5	0/5	0/5
Total	9/60	7/60	10/60

[a] Numerator = number of cultures which survived; denominator = total number of cultures.

In this case the temperature was kept as constant as possible at three different levels, viz., −12° to −15°, −17° to −20°, and −21° to −25° C. Each time the same number of cultures on each medium was used, and they were treated in the same way. The time of exposure to the special temperature was five days. After cooling, the cultures were put at once into the incubator at 18° C., where they were kept for one month to give the fungus opportunity for new development. Transfers were then made to new media. Table 3 gives the results of this experiment.

Not much difference in percentage of survival was obtained at the different low temperatures employed; approximately the same number of cultures resisted −15° C. as −25° C. The great influence of the medium used was again demonstrated. The fungus endures the cold best when grown on manure, next on bogsoil, then on leaf mold. The same result was obtained in the first experiment. It may be added that in another case a culture on clay also resisted −20° C.

Conflicting statements are found in the literature as to the influence of the use of manure on the development of potato blight in the field. In view of the good growth of *P. infestans* on this medium and the strong resistance of the fungus to cold when grown on it, it may be worth while to pay more attention to this point in the future, especially with regard to the influence of manure on the first appearance of blight in the season.

It might be thought that the difference in the resistance of the fungus to cold on the different media was due to a difference of concentration in the decoctions. A correlation between the resistance of fungus mycelium to low temperature and the concentration of the nutrient solution in which they are cultivated was demonstrated by Bartetzko (1), who experimented with four different fungi. In all cases, however, he found a gradual diminution of the percentage of survival corresponding with the lowering of the temperature. The critical temperatures, and the rate at which the percentages decreased, varied with the different concentrations of the solution. He also observed a gradual decrease in percentage of survival in relation to the time of exposure. No such gradual changes were observed in the freezing experiments with *P. infestans*. The relative proportion of cultures that grew after exposure to the three temperatures used, differed only slightly; indeed the lowest gave the highest number of survivals. Neither did the time of exposure exert any observable influence. In Bartetzko's investigations it was the mycelium that resisted the cold in more or less degree according to the concentration of the solution. As has been stated previously, it is probable that the normal mycelium and conidia of *P. infestans* are killed at low temperatures and that it is in some other form that the fungus withstands them. This would explain the behavior of the fungus in the

freezing experiments. It makes no difference in the behavior of these highly resistant organs whether the temperature is – 15° C. or – 25° C. nor whether the exposure to them is long or short.

The oospores are naturally first thought of as being the organs which enable the fungus to remain alive. Manure containing many pieces of straw may hold oospores in abundance. Soils may also contain them in varying number, depending on the presence of silica-containing plant remains. The strange fact remains, however, that the percentage of cultures on rye straw which survived was very small. To obtain further data on this point another experiment was undertaken. This time sixty cultures on different kinds of straw were placed in the refrigerator.

TABLE 4.—*The effect of keeping cultures of Phytophthora infestans dry or moist at a temperature of – 20° C. to – 25° C. for five days*

Medium	Condition of cultures and number which survived	
	Dry	Moist
Rye straw	8[a]/10	0/10
Wheat straw	5/10	0/10
Oat straw	4/10	0/10
Total	17/30	0/30

[a] Numerator = number of cultures which survived; denominator = total number of cultures.

At the same time a new factor was introduced, namely, the influence of moisture and drought. After good development of the fungus, the water was poured out of half the number of tubes. These tubes were kept in a closed vessel containing quick lime, and the lime was renewed from time to time. After one and one-half months all the cultures were subjected to freezing, although those kept in the vessel containing lime were not yet absolutely dry. The cultures were kept in the refrigerator for five days at a temperature of – 20° to – 25° C. After exposure, the dried tubes were filled with sterile water, and then all were placed in the incubator. After a month, transfers were made to new media. The results are shown in table 4.

Very striking is the fact that none of the moist cultures remained alive, while 57 per cent of the dry ones withstood the cold. It is evident that

P. infestans endures low temperatures much better in a dry than in a moist condition. It is a known fact that dry organs are more resistant than moist ones, but it is very remarkable that a fungus like *P. infestans,* which is so dependent on the presence of water for its best development, can endure drought so well. Probably the parts of the fungus which withstand the cold are the same as those which resist drying out. Whether these organs are really only the oospores, is, however, not revealed by the experiment.

TABLE 5.—*The effect of exposing Phytophthora infestans at different stages of development, and growing on different media, to a temperature of −20° C. to −26° C., for five days*

Medium[a]	Form of *Phytophthora infestans* found on the medium in addition to the normal mycelium and conidia	No. of cultures which survived
Potato stem; dead	Very few oogonia, resting forms	$\frac{1^b}{2}$
Potato stem; fresh	Very few oogonia, resting forms	$\frac{1}{2}$
Equisetum; dead	A few oospores; many oogonia, resting forms	$\frac{0}{2}$
Bean stem; dead	Very few oogonia, resting forms	$\frac{1}{2}$
Horse bean stem; dead	Resting forms, chiefly many yellow conidia in clusters	$\frac{1}{2}$
Horse bean pod; dead	None	$\frac{0}{2}$
Horse bean pod; fresh	None	$\frac{0}{2}$
Rhubarb flower stalk; dead	Resting forms	$\frac{0}{2}$
Chrys. leucanthemum; dead	Very few oogonia, resting forms	$\frac{1}{1}$
Beet flower stalk; dead	Very few oogonia, resting forms	$\frac{0}{1}$
Carrot root	Very few resting forms	$\frac{0}{1}$
Mixture of dead plants	Not examined	$\frac{0}{1}$
Pteridium aquilinum; dead	One oospore, moderate number of oogonia, resting forms	$\frac{1}{4}$

[a] All media were desiccated except *Pteridium aquilinum.*

[b] Numerator = number of cultures which survived; denominator = total number of cultures.

The fact that none of the moist cultures remained alive was contrary to what was expected, since oospores must have been present in the thirty cultures on straw in considerable numbers. The next experiment gave similar results.

Cultures on different plant media were subjected to a low temperature. They were all desiccated over quick lime, this time for a period of three months. Consequently the media were absolutely dry before cooling. *Pteridium aquilinum,* only, was added afterwards, and thus was frozen in moist condition. The time of exposure was again five days, the temperature varied from $-20°$ C. to $-26°$ C., the lowest used in the experiments. The tubes were treated subsequently as in the preceding experiments. Results are given in table 5.

In table 5 is added the form of the fungus found in old but rather moist cultures on each specific medium, proving that no special correlation exists between the occurrence of oospores and the survival of the fungus. On the media on which a single oogonium or sometimes not even this was formed, the fungus endured the cold; while on *Equisetum,* the only medium in which distinct oospores develop, both the cultures died. Although it cannot be denied that the presence of oospores may have some influence on the resistance of the fungus to low temperatures, yet some other part of the fungus may be highly resistant as well. The resting forms must be considered. With their thick walls, they are equally as well suited as the oospores to protect the fungus from cold, and the constitution of their protoplasm may have changed to such an extent, as to enable it to better withstand the result of freezing. The gradual drying out of the medium may have favored the development of the resting forms. This experiment proved again the great resistance of *P. infestans* to drought.

It is difficult to decide which of the two forms, the oospores or resting forms, is the more important with regard to resistance to unfavorable conditions, or whether both really fulfill the same function. For the present, it is perhaps best to accept the latter supposition.

The results of all the freezing experiments show that the normal mycelium and conidia of *P. infestans* are not able to resist cold, and, therefore, that special organs are required. These may as well be the oospores as the resting forms. In a dry condition they can withstand low temperatures far better than when moist. These forms can endure a temperature as low as $-26°$ C.

Although special conditions are required for resistance of the fungus to cold, it is not impossible that such conditions occur in nature. On suitable plant remains, oospores may be produced. In autumn weather the unfavorable conditions may exist which stimulate the formation of resting

forms. The great resistance shown in the desiccated condition suggests that a dry period preceding frost may keep the fungus alive better than sudden cold after damp weather.

SUMMARY

1. From the experiments described in the foregoing pages, it is evident that *P. infestans* can live saprophytically. It grows best on old, partially decayed plants. In nearly all soils, therefore, organic parts will be present on which the fungus can feed. In addition to the presence of suitable food, temperature and moisture must be taken into account. Moisture is necessary for the good development of the fungus. It is an important factor in producing the best development and also in renewing growth in the spring. In consequence, it is important in connection with the first foliage infection. The fungus can, however, withstand drought as well as cold. The oospores and the resting forms are the resistant organs; both are probably produced in nature. They are able to endure a temperature of $-20°$ C. to $-26°$ C. for five days, and more easily in a dry than in a moist condition.

2. Theoretically, there is no objection whatever to the hibernation of *P. infestans* in the field. It can remain alive outside the potato plant, and endure cold and drought. Its growth is on the whole rather slow, and abundant only in the presence of moisture. However, the fungus is not easily killed. If *P. infestans* finds the right conditions for overwintering on only one thousandth part of a field, this will be enough to produce a blight epidemic in the following year. Under favorable circumstances in the spring, the fungus will renew its growth.

3. If the tuber is the only source from which infection arises, as DeBary held, a careful inspection of seed potatoes may prevent an epidemic of potato blight, especially in a year following a dry summer. The occurrence of overwintering in the field, however, takes away much of the value of this method of controlling the disease, and provides no other in its place, for with present methods it is impossible to kill the fungus in the soil.

INSTITUUT VOOR PHYTOPATHOLOGIE,
LABORATORY FOR MYCOLOGY AND POTATO RESEARCH,
WAGENINGEN, HOLLAND.

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MOSAIC DISEASES ON DIFFERENTIAL HOSTS[1]

JAMES JOHNSON

WITH PLATES X TO XIV

In connection with studies on the nature of the causal agency of virus diseases of plants, it became of some interest and importance to know more regarding the specificity of the viruses. In the case of the virus diseases of the potato, it seems evident that a number of quite different viruses are concerned. In the cases of mosaics of other plants, however, there has been a tendency in recent researches either to reduce the number on the basis of results from cross-inoculation studies or to give this phase of the problem minor consideration. In the absence of a more satisfactory method, the classification of viruses must be based largely upon the susceptibility of different hosts, the type of symptom produced on these hosts together with the behavior of the viruses toward various other conditions or circumstances which are applicable as tests. The differentiations must naturally be made under such environment, both with respect to external conditions and the condition of the host itself, and in such numbers that infection may be expected to occur. In some respects these requirements are so exacting as to render their usefulness in classification questionable, especially when employed by different individuals under different working conditions. On the other hand, they are not to be considered more unreliable in this respect than are the methods in use for the differentiation of many bacteria and fungi.

The results presented in this paper deal only with certain mosaics of which tobacco is one of the hosts. In another paper it has already been shown that tobacco and other plants are subject to two, and possibly three, different viruses secured from apparently healthy potatoes. These will not, therefore, be treated in this paper, which will be confined to experiments with the well known ordinary tobacco mosaic and four additional ones hitherto undescribed on tobacco.

SOURCES OF VIRUSES USED

The sources of the viruses with which this paper is concerned are as follows:

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture.

A. Ordinary tobacco mosaic: Originally from tobacco fields on Station grounds at Madison. This form occurs commonly in tobacco fields and there can be little question about its type.

B. Cucumber mosaic: From a mosaic cucumber plant furnished by Dr. S. P. Doolittle, Madison (5).

C. Petunia mosaic: From *Petunia violacea* (double flowered variety propagated by cuttings) grown in Horticultural greenhouses at Madison.

D. Speckled tobacco mosaic: From a tobacco leaf with leaf-spot symptoms sent by Mr. L. R. Tehon of the Illinois State Natural History Survey, Urbana, Ill.

E. Mild tobacco mosaic: From tobacco plants sent by Dr. B. W. Tisdale from Quincy, Florida. These plants were said to be affected with leaf-spots of unknown origin.

The ordinary tobacco mosaic has been used in these experiments primarily for comparative purposes. In this connection, however, some interesting points in its behavior have been observed. The designation of the other types of mosaic on tobacco is regarded as tentative and unsatisfactory. The problem of a system of nomenclature for viruses is a difficult one. We are inclined to believe that designation by letters may eventually be as satisfactory as and less confusing than any name based on host relationship or symptoms. The terminology used by geneticists and by workers with vitamins to designate invisible but definite entities might profitably be followed with virus diseases in plants.

The reader should be reminded, therefore, that two different mosaic diseases of the cucumber have already been reported (7) and that tobacco mosaic has been reported as occurring on petunia (2). The term "speckled" tobacco mosaic is only partly descriptive of the disease, but a more serious objection to the use of a descriptive name for this disease on tobacco is that the symptoms on tobacco are often almost identical with those of petunia mosaic, although differing markedly on other hosts.

The term "mild" tobacco mosaic is fairly descriptive since the type of symptom which occurs on young tobacco plants in the greenhouse is distinctly mild as compared with the other types described in this paper, although it may be confused at times with petunia mosaic.

EXPERIMENTAL RESULTS

It is not deemed necessary to describe in detail the methods used in the experimental work. It is believed that the necessary precautions have been observed. It should be stated, however, that we have relied almost entirely upon needle punctures for inoculations. The plants have been

grown in fertile soil in a warm greenhouse (27–32° C.) and for the most part inoculated when very young and in a vigorously growing state.

For the purposes of this paper, the results are adequately summarized in table 1. In some cases it is recognized that the number of inoculations performed is particularly low as far as negative evidence is concerned. It should be stated, however, that comparative inoculations on which final conclusions are drawn were made simultaneously, thereby reducing the possibility of error. In most cases where the symptoms on a host were of a questionable type, comparative inoculations were made from such plants to other differential hosts, so that, in cases where only one positive infection was secured, its type was verified by evidence that accidental infection with other mosaics had not occurred.

In addition to the results presented in table 1 on differential hosts, considerable evidence of other sorts has been obtained to verify these results. This evidence relates particularly to the length of the incubation period, the "infective power" of the virus (as indicated by percentages of infection secured), and the longevity of the virus in a liquid and in a desiccated condition outside of the living host.

The incubation period on tobacco is, for instance, shortest for ordinary tobacco mosaic, somewhat longer for petunia mosaic and speckled tobacco mosaic, and longest for cucumber mosaic and mild tobacco mosaic. The percentage of infection secured with ordinary tobacco mosaic and speckled tobacco mosaic is on the average much higher than that secured with cucumber mosaic, the others being intermediate in this respect. Ordinary tobacco mosaic virus will retain its infectiousness for years in tobacco extract or in dried leaves, whereas cucumber mosaic is limited usually to less than two days in liquid tobacco extract and to two or three weeks in slowly drying leaves. Petunia mosaic is similar to cucumber mosaic in this respect, whereas speckled tobacco mosaic is considerably more resistant to adverse conditions.

The symptoms of the five mosaics on tobacco are on the whole quite distinct on young plants, except in the case of petunia mosaic and speckled tobacco mosaic (Plate X, A), the symptoms of which sometimes appear to be identical. This similarity also seems to hold on certain other hosts. As the plants grow older the symptoms are often less easily differentiated except in the case of mild mosaic, which rarely produces malformation on young tobacco, although on certain other hosts, such as *Physalis*, this virus is comparatively virulent.

Apparently the viruses which are the most difficult to differentiate are petunia mosaic and speckled tobacco mosaic on the one hand, and petunia mosaic and cucumber mosaic on the other. The former two can be dif-

TABLE 1.—*Summarized results of inoculation*[a] *of various mosaic diseases on differential hosts, with most distinctive symptoms*[b] *manifested*

Mosaic	Hosts										
	Tobacco *N. tabacum*	*N. glauca*	*N. glutinosa*	*N. rustica*	Pepper *C. annum*	Tomato *L. esculentum*	*Physalis pubescens*	Petunia *P. violacea*	Nightshade *S. nigrum*	Eggplant *S. melongena*	Pokeweed *P. decandra*
Ordinary tobacco	85 / 84 m, mf, s	10 / 10 s	15 / ± 8 n	10 / 10 n, m, mf, s	8 / 8 m	15 / 15 m, s	5 / 5 m, s	5 / 5 m	5 / 5 m	15 / 10 n	10 / 0
Cucumber	105 / 57 c, m, mf	5 / 2[c] mf	10 / 3[c] m, mf, s	10 / 5 c, mf	8 / 6 m	13 / 7 mf	10 / 4 m, mf	5 / 5 mf, s, n	10 / 1[c] m		20 / 16 m, s, n
"Speckled" tobacco	100 / 91 m, s	10 / 10 m	20 / 0	10 / 9 mf, s, n	10 / 0	10 / 6 m	15 / 2?	10 / 10 mf, s, n	5 / 5 m	16 / 0	10 / 0
Petunia	65 / 40 m, s	10 / 10 m	20 / 6 m, mf	10 / 9 mf, s, n	10 / 0	10 / 4 m	20 / 4 m, mf	15 / 7 m	5 / 5 m	16 / 0	10 / 2 c, n, s
"Mild" tobacco	45 / 24 m	5 / 0	15 / 7 c, s, n	10 / 4 m, mf	5 / 1[c] m	5 / 4 m	5 / 5 c, s	5 / 0	5 / 1[c] m	5 / 0	10 / 0

[a] Upper figure is number of plants inoculated; lower figure number of plants infected.

[b] C = chlorosis; M = mottling; N = necrosis; Mf = malformation; S = stunting.

[c] Type verified by inoculation.

ferentiated on *N. glutinosa,* petunia (Plate XII), pokeweed and probably on *Physalis,* as well as by their comparative longevity outside of the host. The two latter are best differentiated on *N. rustica,* tomato, or petunia.

Other solanaceous hosts have been tried, but on the whole have not been used sufficiently to warrant including them in table 1. It should be stated, however, that tobacco mosaic was found to produce symptoms on *Solanum rostratum* (new host), while it failed to give mottling on *Datura stramonium,* although it produced striking lesions on the stems of young plants when applied to them with slight wounding. Similar lesions were produced on various other plants, including potato and pepper. In the case of young plants of *N. glutinosa* and *N. rustica,* this virus is frequently capable of killing the plants as a result of stem lesions, even though, peculiarly enough, in the case of *N. glutinosa,* the virus is not apparently present in the foliage. Tobacco mosaic produced distinct stunting but little, if any, mottling on *N. glauca.* It affects egg plant only with difficulty, mottling rarely developing, although stunting and stem or leaf necrosis may be very striking on young plants.

Cucumber mosaic usually produces larger chlorotic areas on tobacco than tobacco mosaic, and these areas are frequently more distinct near the tips of the young leaves. The symptoms of cucumber mosaic on *N. glutinosa* are especially striking with respect to mottling and malformation. On tomato, cucumber mosaic frequently produces the characteristic foliage malformation sometimes referred to as the "maiden-hair fern" type (Plate X, C). Ordinary tobacco mosaic has not yet produced this type of symptom on tomato in our experiments. As compared with the other species studied, petunia and pokeweed appear to be especially susceptible to cucumber mosaic. Speckled tobacco mosaic also is virulent on petunia. In one series of inoculations, all of the five plants were killed. Petunia mosaic, on the other hand, seemed to be only mildly effective on the petunia plants used. It should be stated, however, that the variety used was heterozygous in nature and not the same as that from which the mosaic originally came.

The behavior of the speckled and petunia mosaics on *N. glauca* is interesting in that the symptoms of mottling are very faint and sometimes accompanied by a scattered purplish tint as compared with the controls.

The mild mosaic on tobacco is so readily masked that symptoms often apparently disappear and reappear intermittently over short periods of time, depending apparently on minor environmental variations. On *N. rustica,* however, decided malformation may occur, and young plants of *N. glutinosa* may be killed or stunted without occurrence of mottling. While the data on *Physalis* is limited, the symptoms were so marked that there can be little question but that it should be regarded as a good differential host for mild mosaic (Plate XI).

DISCUSSION

The results presented are believed to justify the conclusion that we have been dealing in these experiments with five separate viruses. It has been shown in another paper also that two and possibly three additional ones have been secured from apparently healthy potatoes. There are in all probability several more viruses which will eventually be found to attack tobacco and other solanaceous plants, since we have not yet made a preliminary survey of this field of investigation, utilizing only those specimens which were conveniently at hand during the winter months or which were sent to us for determination by other workers.[2] It might be argued that a virus is not a definite entity but is so modified by its hosts or other conditions that cross-inoculation experiments of this sort are of little actual significance. Our experience with these viruses, carrying them through several generations of the same and different hosts, is quite contrary to this suggestion, although it is admitted that this important possibility merits more special consideration than we have been able to devote to it.

It is believed that the results presented here suggest a reasonable explanation for some of the contradictory results secured by some workers with tobacco mosaic. This may best be illustrated by the experience of certain investigators with the mosaic disease of *N. glutinosa*. Allard (3) found this species (the *N. viscosum* of Allard's paper) immune from tobacco masaic, but reported it to be affected with a mosaic distinct from tobacco mosaic. Walker (9) and later Elmer (6) question Allard's original conclusion. Our experiments on this subject indicate that Allard was essentially correct in his interpretation. Tobacco mosaic apparently is not capable of acting as a systemic invader of *N. glutinosa*, although it may cause very marked necrosis of young stems; the virus is apparently not present in the leaves (Plate XIII, A). Many other plants are affected by ordinary tobacco mosaic in a similar manner, but such lesions cannot be said to represent true susceptibility to mosaic.

It has been shown in this paper that cucumber mosaic and petunia mosaic produce typical symptoms of mosaic mottling and malformation on *N. glutinosa* (Plate XIII, B), and it is not unlikely that one of these—most likely the former—is Allard's mosaic on *N. glutinosa*, as well as the one used by Walker and by Elmer. We have, in fact, considerable evidence in that direction, which is not deemed sufficiently important to be presented in detail here.

The necrotic lesions produced on stems of various plants by tobacco mosaic is especially interesting and merits special study. This occurs ap-

[2] Since this paper was written, at least three additional viruses have been found to occur on tobacco.

parently on species which are true hosts as well as on others which are not true hosts. These lesions have been observed principally upon potato, *Datura*, pepper, *N. glutinosa* and *N. rustica*.

N. glauca has been regarded as a typical "carrier" of tobacco mosaic, *i.e.*, carrying the virus without itself showing symptoms. *N. glauca*, however, may become very markedly stunted by tobacco mosaic, while all other symptoms usually are lacking (Plate XIV). *N. glauca* is also susceptible to cucumber mosaic, which produces distinct mottling and malformation, as well as to other virus diseases producing more marked leaf symptoms than does tobacco mosaic. Our experience with this and other species indicates that our understanding of true virus "carriers" (1, 8), as distinct from masking and other conditions, is so limited and uncertain as not to warrant the use of the term "carriers" in a sense distinct from plants with masked symptoms.

The recent work of Elmer (6) in particular tends towards the conclusion that little specificity is shown by mosaics from a wide range of hosts. As far as known, however, it has not yet been definitely shown that ordinary tobacco mosaic has been transmitted to hosts outside of the solanaceous family. On the other hand, cucumber mosaic is apparently capable of infecting plants in widely different families. The results presented in this paper show that tobacco, like the potato, may be host to a number of different virus diseases. These observations merit careful consideration in any theory developed as to the nature of the causal agency of virus diseases in plants.

SUMMARY

1. Tobacco, tomato and other solanaceous plants, aside from the potato, have been generally believed to be affected with only one mosaic disease, namely, tobacco mosaic. This paper presents evidence that at least 5 distinct mosaics may occur on various species of this family in addition to other virus diseases not described in this paper.

2. The four additional mosaics are: cucumber mosaic, petunia mosaic, "speckled" tobacco mosaic and "mild" tobacco mosaic.

3. These mosaic diseases are shown to be different from tobacco mosaic and from each other primarily through their behavior on differential hosts.

4. It is believed that these results demonstrate that it is important in future researches with mosaic to differentiate and describe more specifically the type of mosaic used in experimental work.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

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EXPLANATION OF PLATES

PLATE X

A. "Speckled" tobacco mosaic on tobacco. This type of mottling is somewhat characteristic for this mosaic as compared with ordinary tobacco mosaic.

B. Tobacco mosaic. A common type of mottling. The variation in symptoms is so large, however, that "speckled" and ordinary tobacco mosaic cannot always be distinguished on tobacco.

C. Upper and lower leaves from tomato plant infected with cucumber mosaic. Note type of malformation in upper leaf and chlorosis and stunting in lower leaf. This symptom has been fairly distinctive under the conditions of the experiments described in this paper.

D. Upper and lower leaves of healthy tomato plant for comparison with C.

PLATE XI

A. Healthy controls of *Physalis* and *N. glutinosa*.

B. *Physalis* and *N. glutinosa* plants infected with the "mild" type of tobacco mosaic. Marked stunting and chlorosis occurs on *Physalis*, but only moderate stunting and chlorosis on *N. glutinosa*.

C. *Physalis* and *N. glutinosa* plants infected with petunia mosaic. Note stunting, malformation and mottling on both hosts in comparison with controls (A).

PLATE XII

A comparison of petunia mosaic and "speckled" mosaic.

A. *N. rustica* inoculated with petunia mosaic.

B. *N. rustica* inoculated with "speckled" mosaic.

C. Petunia inoculated with petunia mosaic.

D. Petunia inoculated with speckled mosaic.

E. *N. glutinosa* inoculated with petunia mosaic.

F. *N. glutinosa* inoculated with "speckled" mosaic.

Note that while the symptoms are apparently identical on *N. rustica*, they differ markedly on the other plants.

PLATE XIII

A. *N. glutinosa* inoculated with ordinary tobacco mosaic.

B. *N. glutinosa* inoculated with cucumber mosaic.

C. Control, not inoculated.

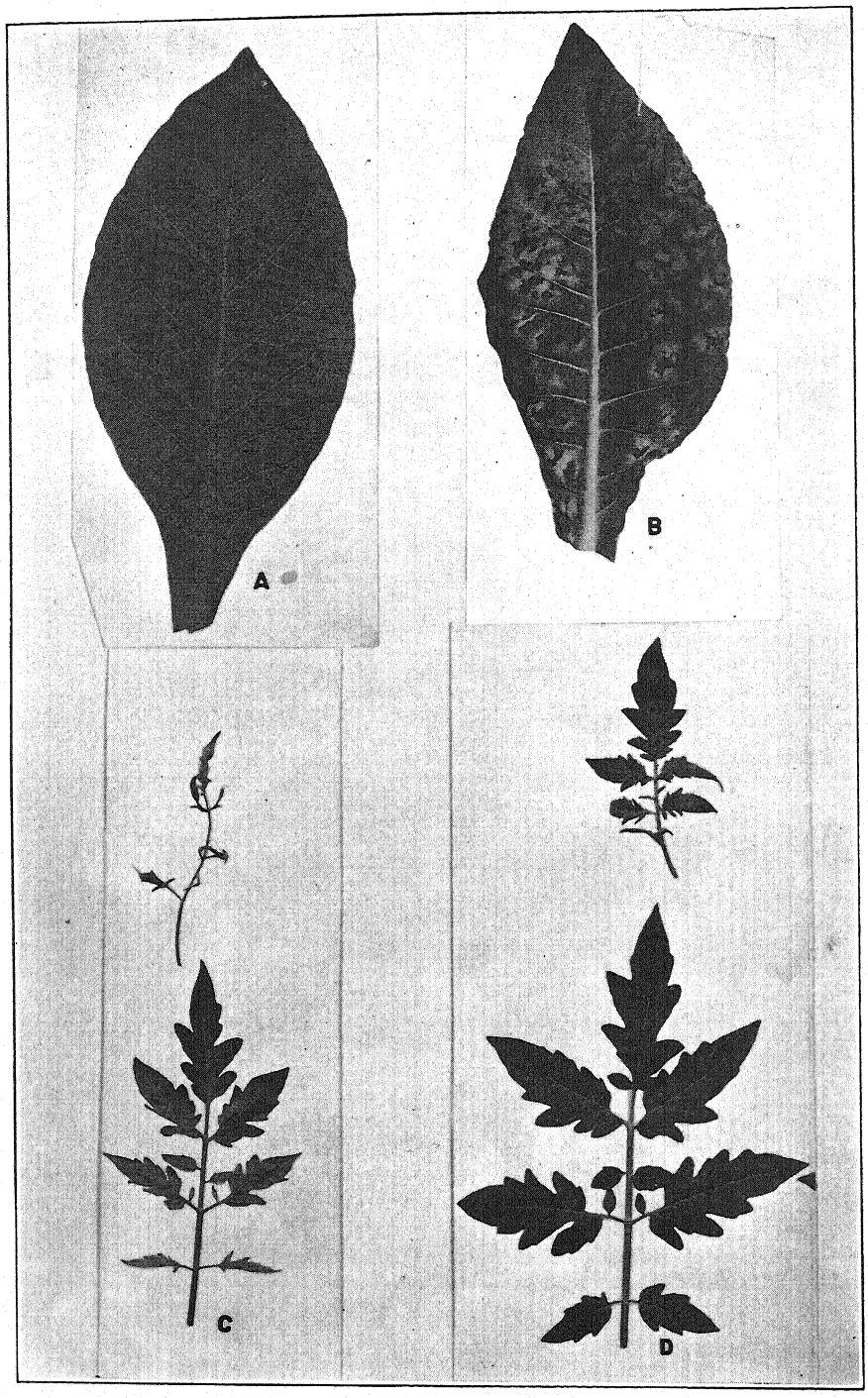
D. *N. glutinosa* inoculated with "ring-spot" virus secured from apparently healthy potatoes.

Ordinary tobacco mosaic frequently kills young *N. glutinosa* plants due to necrotic action on stems, yet the virus is apparently not systemic in the leaves. The type of virus disease shown in D illustrates the distinctiveness of still other types besides those described in this paper.

PLATE XIV

A. *N. glauca,* uninoculated control.

B. *N. glauca* inoculated with ordinary tobacco mosaic showing faint chlorosis of leaves but more particularly marked stunting of growth.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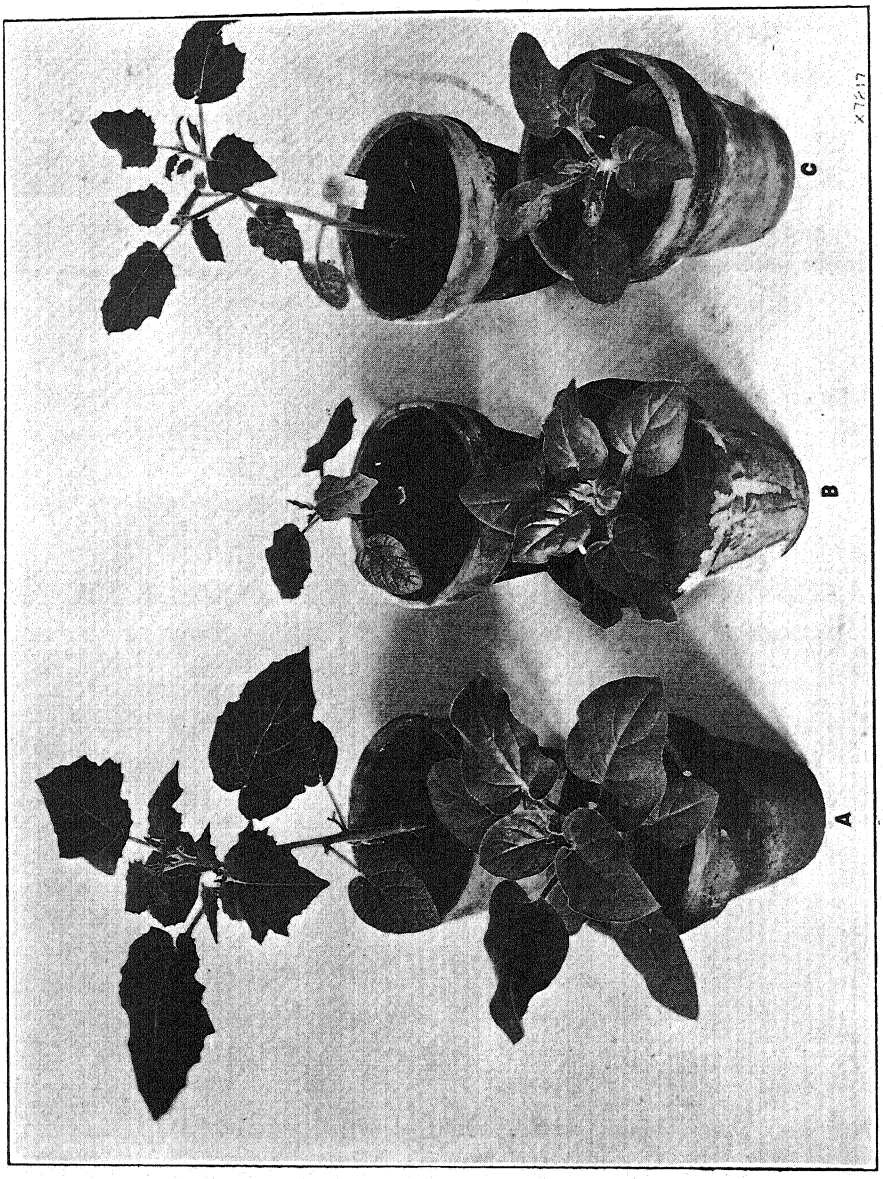
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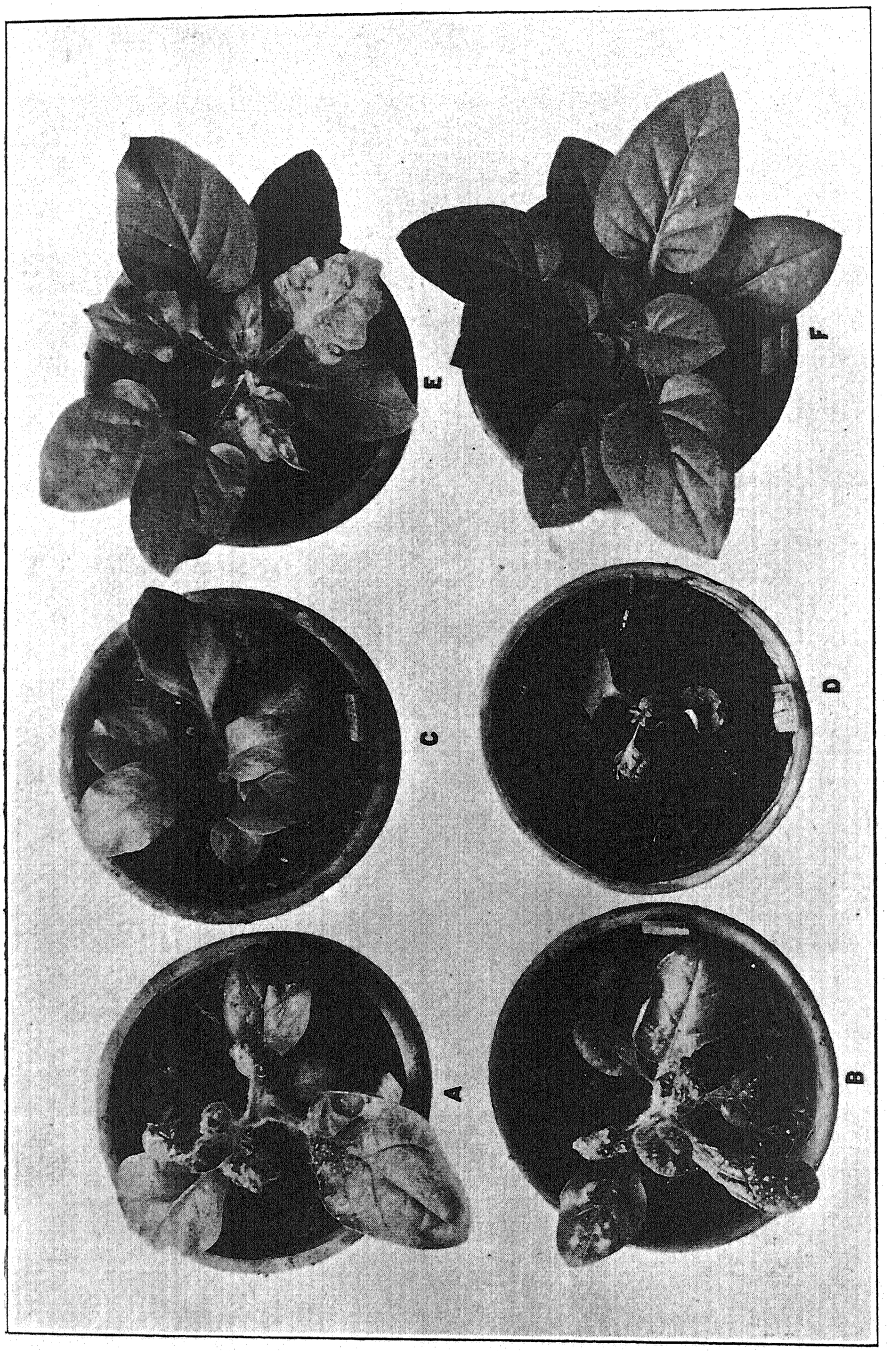
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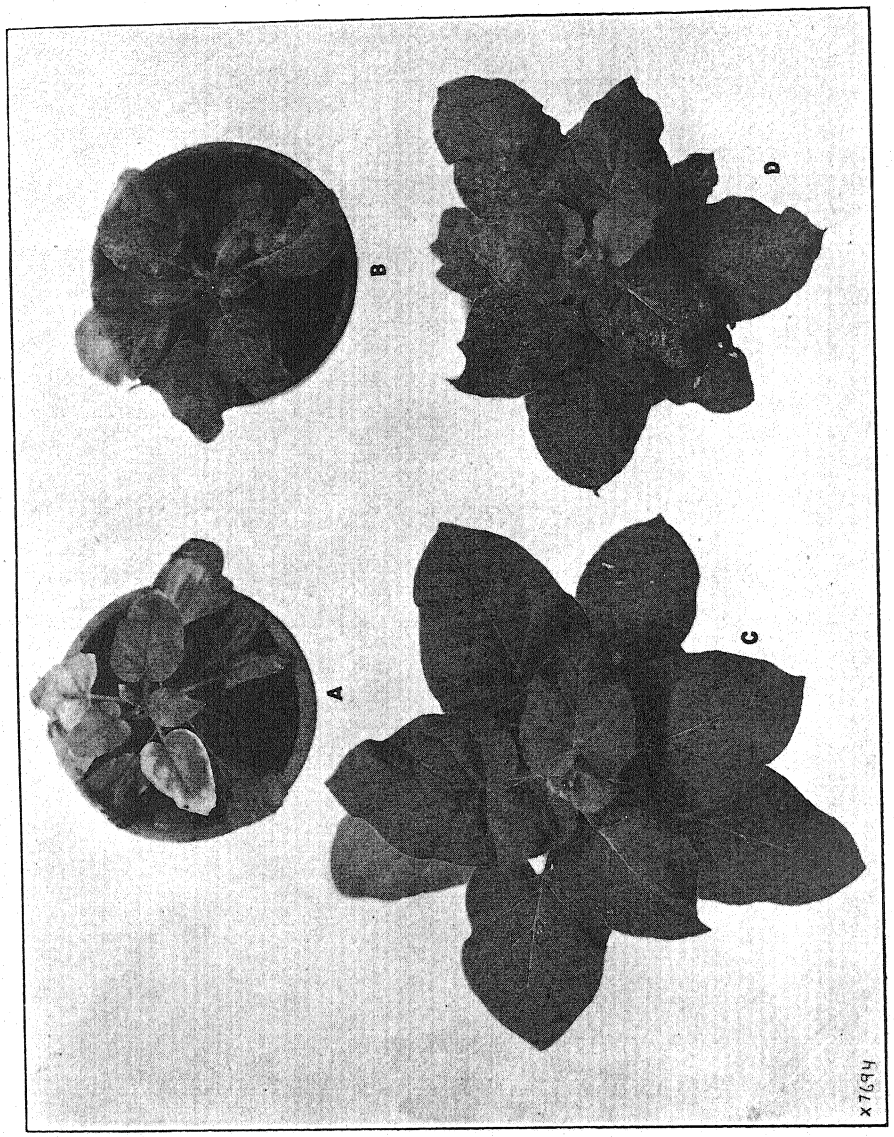
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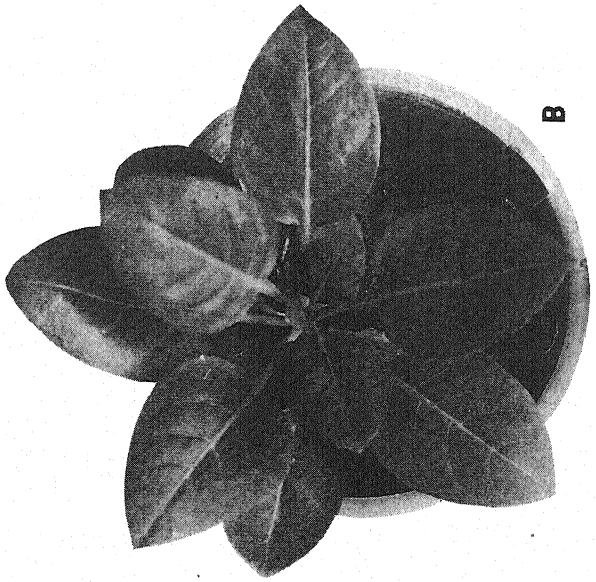
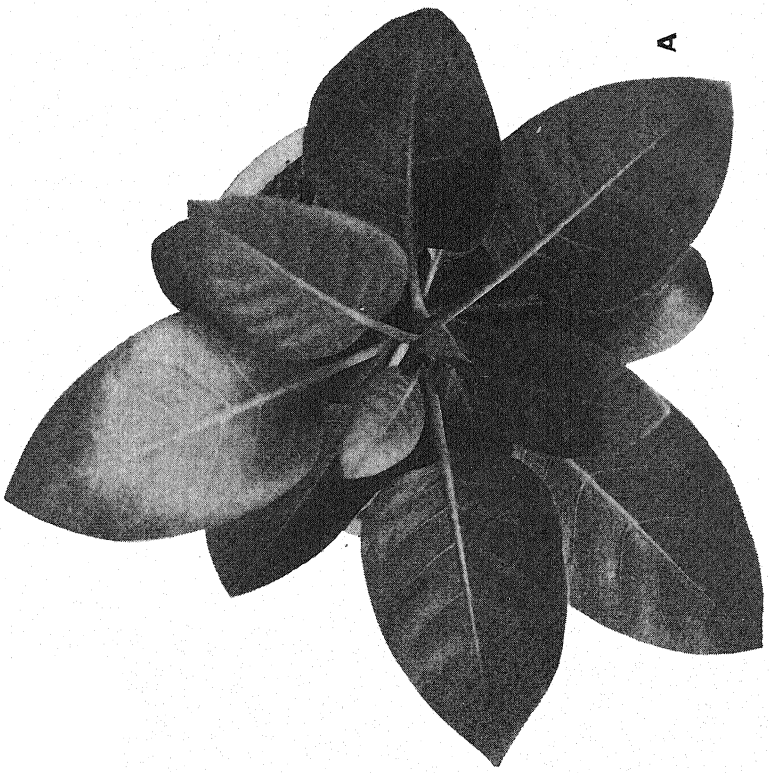
PLATE XIV





PHYTOPATHOLOGY

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THE RELATION OF THE SEED-CORN MAGGOT (PHORBIA FUSCICEPS ZETT.) TO THE SPREAD AND DEVELOPMENT OF POTATO BLACKLEG IN MINNESOTA[1]

J. G. LEACH

INTRODUCTION

It has been assumed generally by plant pathologists that the pathogene of potato blackleg overwinters in, and is spread only by, infected tubers. Morse (22), in 1917, stated that "Observations in Maine indicate that under the climatic conditions which exist there infected seed potatoes are the sole source of infection and distribution . . ." The only other possible source of infection that has received much attention is the soil.

Rosenbaum and Ramsey (27) could find no evidence to indicate that the pathogene could survive in the soil, or in diseased tubers in the soil, in Aroostook County, Maine, or at Norfolk, Virginia. They placed both naturally and artificially infected tubers in the soil in the fall, and planted selected and disinfected tubers the following spring, in the rows where the diseased tubers had been planted the previous fall. No blackleg developed, and they were unable to reisolate the pathogene from the soil.

Ramsey (25) inoculated one-half-pint jars of soil with 15 cc. of a broth culture of the blackleg pathogene and buried them in the soil over winter, but he was unable to reisolate the organism by dilution cultures the following spring. He also watered growing potato plants heavily with broth cultures of the pathogene. In other cases the soil was inoculated by incorporating decayed tubers, some of which were placed in direct contact with the seed piece. Only one plant of the many inoculated developed the disease. From these experiments it was concluded that "Unless the seed piece is infected at planting time, there is little chance that uninjured plants will contract the disease even though the causal organism is washed about the stem and root system."

[1] Published with the approval of the Director as paper No. 583, Journal Series of the Minnesota Agricultural Experiment Station, St. Paul, Minn.

Pethybridge (24) in Ireland, and Murphy (23) in Canada, held similar views.

On the other hand, von Hegyi (14), working in Polish Galicia and Prussian Silesia, found such a close correlation between blackleg and wire worm attack that he held biting insects to be a necessary factor for entry of the parasite. Little attention has been given by subsequent workers to this insect or other insects as possible means of spread of the disease.

Jennison (16) states that "The larvae of insects have been found by the writer working in and on the affected tissues, but there was no positive evidence that they were active agents in the dispersal of the disease." No experiments with the insects were mentioned.

Kotila and Coons (18) showed that in Michigan under certain conditions the bacteria overwintered in tubers remaining in the soil. They also showed that the blackleg pathogene was present in the soil surrounding diseased plants, but concluded that "It does not, however, seem capable of existing there for any considerable length of time."

Murphy (23) states the prevailing attitude of pathologists toward the question as follows:

"Although there seems to be considerable reluctance in finally accepting the view that this disease is not, for practical purposes, harbored or spread by the soil, failure to bring about infection from this source, and the success of control measures applied to the tubers, leave no other alternative open. The skepticism which has prevailed as to the absence of soil infection is to be traced to three, or probably four, factors: the failure to account for certain widespread outbreaks in plants produced from seed tubers presumed to be practically uninfected; the want of any entirely satisfactory theory of hibernation in the tubers; the failure of attacks to develop in certain cases from infected seed potatoes under conditions presumed favorable; and the possible confusing of fusarium-wilt with blackleg."

Since the summer of 1920 the writer has had occasion to investigate numerous outbreaks of blackleg in Minnesota which could not be satisfactorily explained on the basis of our present knowledge. Many of the fields investigated had been planted with certified seed from fields known to be free from blackleg, and the seed had been properly disinfected and planted with the best of care. It was thought at first that the disease might have been spread from a few infected tubers in the cutting operations, for Morse (21) states that "There is every reason to believe that in cutting seed the disease may be spread from diseased to healthy seed pieces by contact of their freshly cut, moist surfaces and less frequently from the hands of the operator or through the medium of the knives used." However, he cites no experiments to support the statement.

ATTEMPTS TO PRODUCE THE DISEASE BY SEED PIECE INOCULATION

In the spring of 1922, experiments were planned to determine the possibility of spread of the disease by seed piece contamination during the cutting operation. A quantity of tubers were inoculated with a virulent culture of the blackleg pathogene which previously had been tested by inoculating stems of growing plants in the greenhouse. After incubation in a moist chamber for several days, the tubers were completely decayed. The cut surfaces of sixty freshly cut seed pieces were thoroughly smeared with the rotted tubers. These in turn were incubated for twenty-four hours in a moist chamber and then planted at University Farm. Much to the surprise of the writer, not a single case of blackleg developed.

The following year this experiment was repeated on a larger scale, using a more positive means of inoculation. About four hundred freshly cut

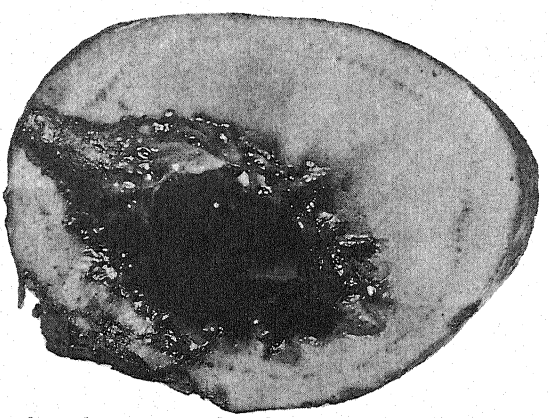

FIG. 1. A section through a potato tuber three days after inoculation with the blackleg pathogene by the method described in the text: typical of tubers used in the experiments on seed piece inoculation.

seed pieces were placed in large crocks, sprayed with a broth culture of the pathogene,[2] and incubated until the decay had extended to an average depth of approximately one-fourth inch. Some of the seed pieces were punctured through the skin and decay was also started at these points. Some of the tubers were planted on mineral soil, and some on peat soil, where a variation in the water level from one to five feet was maintained. Again not a single case of blackleg developed, although a few of the badly rotted seed pieces decayed completely before a sprout could be sent out.

[2] In this and all subsequent inoculation experiments, two cultures of the pathogene were used. One was isolated and tested by the writer, and the second was received from Dr. Erwin F. Smith under the name "*Bacillus phytophthorus* Appel I 1906." Both cultures were strongly pathogenic to potato stems and tubers.

Many of the seed pieces were later dug up and examined, and, in every case, it was found that the decay had been effectively walled off with a thick layer of cork cells. These results would indicate strongly that the disease was not commonly spread in this way. However, it was thought that, since the decay extended from the surface inward and was in direct contact with the soil, some detrimental action of the soil might be responsible for the failure of the pathogene to develop. Accordingly, the experiment was repeated in 1924, using a different method of inoculation. A hole about one inch deep was bored into the tuber with a one-fourth-inch wood

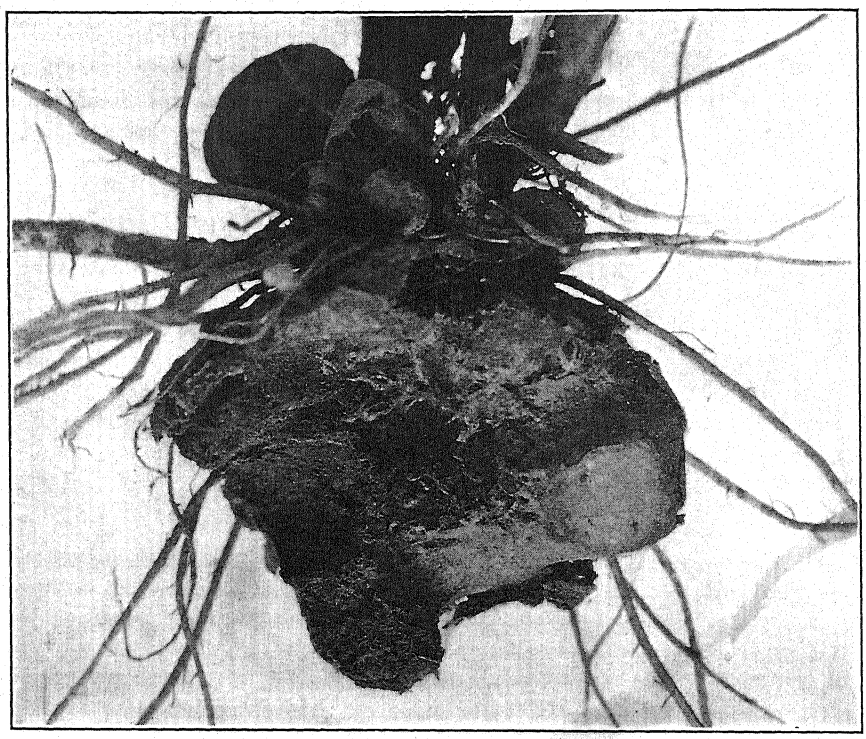

FIG. 2. A seed piece, which had been inoculated with the blackleg pathogene, dug up more than two months after planting in the field. The decayed portion had been walled off entirely by a layer of cork. The outline of the decayed cavity and the inoculation hole are still visible.

bit. The hole was almost filled with a broth culture of the pathogene and then plugged with a tightly fitting plug cut with a cork borer from another tuber. After incubation for three days, the decay was found to be developing rapidly (Fig. 1). The tubers were planted without disturbing the plug. The results were the same as in previous years. Not a single case

of blackleg developed, and, on examination, it was seen that the seed piece had effectively corked off the decay (Figs. 2 and 3, Plate XV, l, m, and n). These experiments seem to indicate conclusively that seed piece infection at cutting time is not an important factor in the development of the disease, providing the seed piece is planted under conditions not inhibitory to cork formation.

THE ASSOCIATION OF THE SEED-CORN MAGGOT WITH BLACKLEG PLANTS

In the summer of 1923, while seeking further for an explanation of the outbreaks of blackleg from seed apparently free from the disease, a large number of diseased plants were dug up, seed piece and all, and carefully examined. Most of the observations were made fairly late in the season, after the disease was well advanced. In many plants a number of small white maggots were found working in the stems. In a few cases the maggots were found in the old seed pieces, but in most cases only fragments of the seed piece were left. It was impossible at this time to tell whether the maggots were of any significance or whether they were merely feeding on the plants which had already started to decay. However, a number of the maggots were collected and reared to maturity. Several different

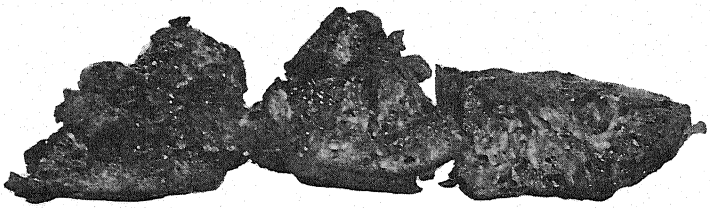

FIG. 3. Seed pieces infested with seed-corn maggots. These seed pieces had been planted about thirty days, and the plants were just beginning to show typical symptoms of blackleg. Note the slimy burrows. The maggots are practically full grown and almost ready to enter the soil and pupate.

species were obtained, but most prevalent was the seed-corn maggot (*Phorbia fusciceps* Zett.).[3] Inasmuch as this fly is recognized as a parasitic insect on a number of crop plants, it appeared that its presence in potato plants affected with blackleg might be more than incidental.

In the spring of 1924 a very thorough study was made of the relation of the insect to the development of blackleg. Unfortunately, on account

[3] These first specimens were kindly identified by Dr. J. R. Eyer, formerly assistant in entomology at the University of Minnesota. Later specimens were sent to Dr. J. M. Aldrich of the National Museum and to Dr. O. A. Johannsen of Cornell University, both of whom verified the identification. The insect is probably more properly called *Hylemyia ciliicrura* Rond, but, since it is better known in American literature on economic entomology as *Phorbia fusciceps,* this name has been used.

of other duties, the writer was unable to make any extensive observations at the time of planting. On June 26, shortly after the potato plants had appeared above ground in the Red River Valley, a field laboratory was established at the Northwest School of Agriculture at Crookston, Minnesota. The first infected plants were observed on June 29 in a field of Early Ohio potatoes, in which the healthy plants were about six inches tall. About one per cent of the plants were beginning to show the characteristic symptoms of the disease. In the next three days more than a hundred diseased plants were dug up and examined. Maggots were found in the seed pieces of all but six plants (Fig. 3). The maggots varied in length from four to seven mm., and some were beginning to pupate. A puparium was found in the soil near one of the six plants. The other five seed pieces yielded no maggots, although it is possible that puparia may have been overlooked. Two days later, when a more careful examination of the surrounding soil was being made, several puparia and a few pupal skins were found.

During the next three weeks more than 1,000 plants were examined. The plants came from about fifty different fields in three counties. Each successive day the number of puparia in proportion to maggots increased. Pupation was at its height by July 4, and many adults were emerging, as indicated by the pupal cases found.

Several hundred maggots and puparia were collected and put in bottles of soil which were placed in a screen cage in the laboratory. Practically all of these eventually developed into adult flies. Of more than three hundred flies obtained in this way, all but seven were identified as the seed-corn maggot fly.

THE SEED-CORN MAGGOT

The seed-corn maggot, which has been somewhat inappropriately named, is closely related to the better-known cabbage maggot (*Phorbia brassicae* Bouche). The adult insect is a fly slightly smaller than the common house fly (Fig. 8). The larvae are known to be parasitic on a number of crop plants. In a brief survey of the literature, reports of injury on corn, beans, peas, turnips, cabbage, radishes, onions, beets, potatoes, hedge mustard (8), tomatoes (30), and wheat (28) were found. In New Jersey the insect is considered a serious pest of beans (13). Lugger (20), in 1896, reported a serious outbreak of the insect on beans in Minnesota. During the summer of 1924, members of the Division of Economic Entomology of the University of Minnesota observed several severe infestations of beans. It is said to be distributed generally throughout the United States, Canada, and Europe (5). It has been reported from decaying potato plants or "seed potatoes" from New York (28), Louisiana (30), Florida (29), Idaho, Maryland, and Virginia (4).

Sellards (29) reports the insect very destructive to potatoes in Florida in 1905, and describes its attack as follows:

"The decay of the potatoes was found to be more severe on the low wet lands. The maggots were present on the higher lands, although in smaller numbers. The attack on the potatoes is made from the cut surface. The maggots seem to prefer decaying potatoes, but attack perfectly sound ones. Starting from the cut surface, tunnels are run in various directions through all parts of the potato. In some cases the potatoes were found much decayed and a mass of maggots, hardly anything of the potato remaining but the outer skin."

Hylemyia trichodoctyla Rond, a species almost indistinguishable from the seed-corn maggot and which is known as the seed-potato maggot, has been reported from potato plants in Aroostook County, Maine (17) and from turnips in Ontario (12). The writer has not recognized this species in Minnesota.

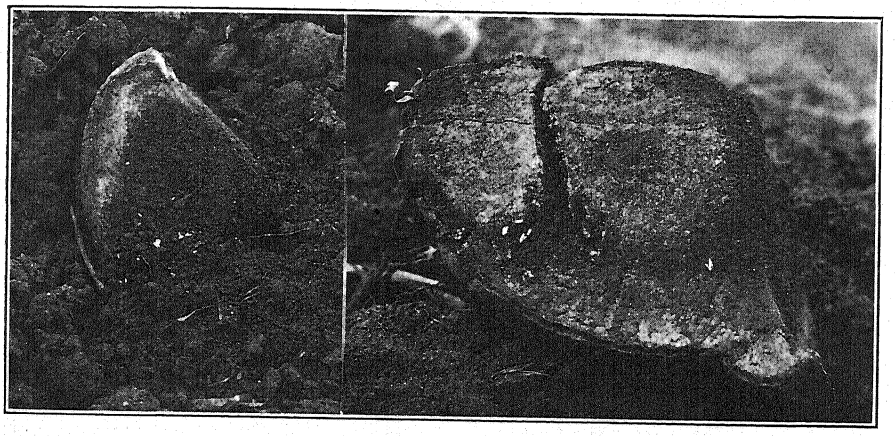

FIG. 4. Eggs of the seed-corn maggot on, and in the soil near, potato seed pieces. Photographed three days after planting. These seed pieces had been planted by machine in the usual way, but were incompletely covered. The eggs were mostly on the under side of the seed pieces, which were turned over before the eggs were discovered.

LIFE HISTORY

Eggs.—Like the cabbage-maggot fly, the adult female of the seed-corn maggot deposits eggs on, or in, the soil near freshly planted seeds or young sprouting seedlings. The early observations of the writer (19), which were made several weeks after planting time in 1924, led him to think that the eggs were deposited on potato seed pieces before planting, but further observations in 1925 showed quite clearly that the eggs are deposited most frequently on, or in, the soil near seed pieces after planting (Fig. 4), and

even after the sprouts have appeared above the ground. Evidence of egg deposition in the soil before the seeds of beans were planted has been reported from New Jersey (13). The eggs are small, slightly less than 1 mm. in length, but may be seen readily with the naked eye. They are slightly curved, white, and distinctly reticulate (Plate XV, b and c). When freshly deposited, they are covered with a sticky fluid, which causes them

FIG. 5. Soil cracks along the top of a potato row three days after planting. Eggs of the seed-corn maggot were found several inches below the surface of the soil in these cracks. The photographs in fig. 4 and this one were taken in the same field on the same day. There was more than 10 per cent blackleg in the field when observed about two months later.

to adhere to the soil particles or anything with which they may come in contact. The writer has frequently observed the adult female deposit eggs in the soil near exposed seed pieces. With her hind legs the female pushes aside small clods of earth until her ovipositor is several mm. beneath the

surface, and the eggs then are deposited, usually in direct contact with moist soil.

In 1925 the adult flies were very abundant as early as May 2 in certain potato fields in northern Minnesota. Potato planting did not begin in this section until about May 10. The first eggs observed were found on May 13, and the height of the egg-laying period was reached about the 25th and 26th of May. During this period many eggs were obtained for experimental purposes by placing freshly cut seed pieces on the surface of the soil in potato fields and leaving them exposed for twenty-four hours. On May 21 more than 500 eggs were obtained from the soil in immediate contact with less than 100 seed pieces which had been exposed not more than twenty-six hours. In many instances, egg deposition has been observed in less than ten minutes after exposing the seed pieces.

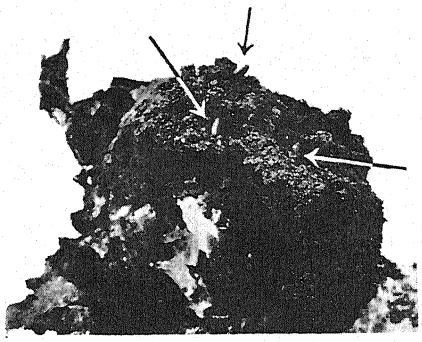

FIG. 6. Three puparia of the seed-corn maggot in the soil, adhering to a decayed seed piece from a blackleg-infected plant. These puparia were close to the seed piece, although they are frequently found several inches away.

The soil in the Red River Valley contains a great deal of colloidal material and is very subject to cracking. Almost any freshly turned soil cracks abundantly upon drying, although the soil may remain flocculent and friable. It is not uncommon for rather large cracks to develop along the ridge of soil left by the potato planter. These cracks are frequently several inches deep, and are directly over the planted seed pieces (Fig. 5). Flies were observed near these cracks, and, on careful examination, eggs were found as deep as four inches beneath the surface of the soil. Eggs also have been found several inches beneath the surface of the soil in cracks made by the sprouting seed piece as it breaks through the ground.

Larvae.—The eggs hatch in about three days after they are deposited. The maggot, when it emerges, is nearly transparent and only slightly longer than the egg, but it is able to move about rapidly in search of food. The action of freshly-hatched maggots was observed by placing one-half of a

sound, freshly-cut tuber and several eggs in contact with the wall of a glass jar and filling the jar with moist soil. Most of the eggs hatched within twenty-four hours. The young maggots had no difficulty in finding the seed piece, although some of the eggs were more than an inch from the tuber. For the first twenty-four hours after hatching, the maggots crawled over the cut surface of the tuber. At the end of this period a profuse growth of bacteria was evident. During the next two days most of the maggots disappeared into the interior of the tuber, which was completely decayed in less than a week. The remaining half of the same tuber, kept under similar conditions but not attacked by maggots, remained healthy.

The mouth of the maggot is equipped with two black, sharp, claw-shaped structures (Plate XV, j), with which it is able to tear apart the tissues of the

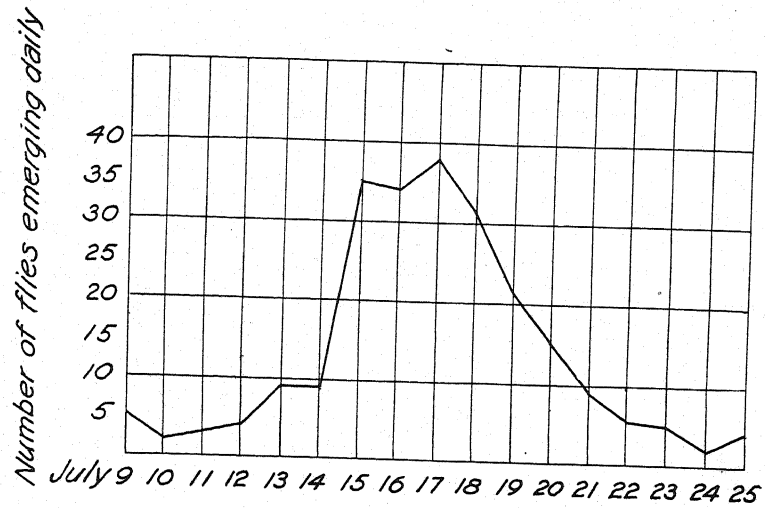

FIG. 7. A graph showing the time of emergence of 231 adult seed-corn-maggot flies from puparia collected from blackleg-infected plants in 1924.

seed piece, and which no doubt serve as very effective means of inoculating the seed piece with bacteria. It is obvious that, with maggots present, any cork laid down by the tuber would be quickly destroyed and would not be effective in checking the decay.

Puparia.—The maggots enlarge very rapidly, and in from fifteen to twenty-five days reach a maximum length of about 7 or 8 mm. (Plate XV, f). When this size is reached, they leave the decayed seed piece, enter the soil, and pupate (Fig. 6). Puparia frequently are found four or five inches from the seed piece, although most of them remain within a radius of three inches or less. In nature, pupation begins about one month after the pota-

toes are planted, and frequently it is under way when the first symptoms of blackleg are evident. In a field planted on the 28th of May, 1924, the first blackleg-infected plants were observed on June 29, when the normal plants were from six to eight inches high. Puparia also were found at this time; and, by July 4, puparia were more abundant than larvae. Many plants which manifested the first symptoms of blackleg after this date were examined, and almost invariably it was found that pupation already had occurred. This early pupation may account for the fact that the insect has been so commonly overlooked.

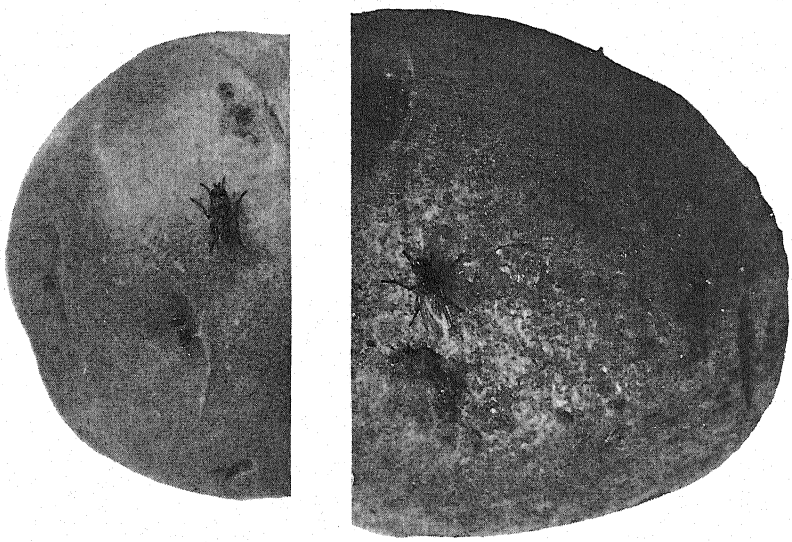

FIG. 8. Flies of the seed-corn maggot (female, left; male, right) resting on potato tubers—a very common sight in Minnesota potato fields at planting time.

The puparia (Plate XV, h) are from 4 to 5 mm. in length, and vary in color from light brown to dark reddish-brown. The duration of this stage varies from seven to fourteen days. Fig. 7 shows graphically the period of emergence of 231 flies from puparia collected in 1924.

Adult flies.—The adults, which for some unknown reason emerge from the puparia mostly at night, are able to work their way through several inches of soil. When freshly emerged, they are lighter in color than normal, and their wings are not unfolded. Within a few hours, however, they take on their normal gray color and their wings unfold. Adult flies are shown in figure 8, and Plate XV, a and d. A detailed description of the adults will not be given here, as it can be found readily in entomological literature. Mention should be made, however, of the usual means of distinguishing them from other closely related species. On the tibia (middle

segment) of each hind leg of the male, there is a row of from 21 to 24 short, slightly curved bristles (Plate XV, g). These bristles distinguish the seed-corn maggot from all other species of the same genus, except the seed-potato maggot. It is often very difficult to distinguish the females from those of closely related species.

FIG. 9. Secondary infection of blackleg disseminated and inoculated by the seed-corn maggot. A young maggot was found working at the base of the cluster of roots indicated by the arrow. The infection was entirely confined to the cortex. The pith was not affected. The dark spot at the base of the stem is a lesion caused by *Rhizoctonia solani*. The negative was much under-exposed.

Broods.—The number of broods of this insect are not definitely known (8). At least two broods have been observed by the writer in Minnesota. The second brood eggs are deposited most frequently near the ground line of potato plants affected with blackleg, and the maggots develop in the stems of such plants. In only a few cases have the maggots of the first brood been observed to pass upward into the stems. A considerable number of the second brood eggs are deposited also at the base of the healthy potato plants, which later become diseased. Several well marked cases of blackleg have been observed which were without doubt due to this type of secondary infection (Fig. 9).

The insects are supposed to hibernate as puparia in the soil. Evidence of this was observed in the early spring of 1924, when adult flies were very abundant in fields which were known to have been heavily infested the previous year, and none could be found in neighboring fields known to

have been free from blackleg. Observations indicate that the insect usually does not hibernate in potato cellars or piles of refuse potatoes. Many species of scavenger flies have been observed breeding in piles of refuse potatoes, but the seed-corn maggot never has been found developing in such places.

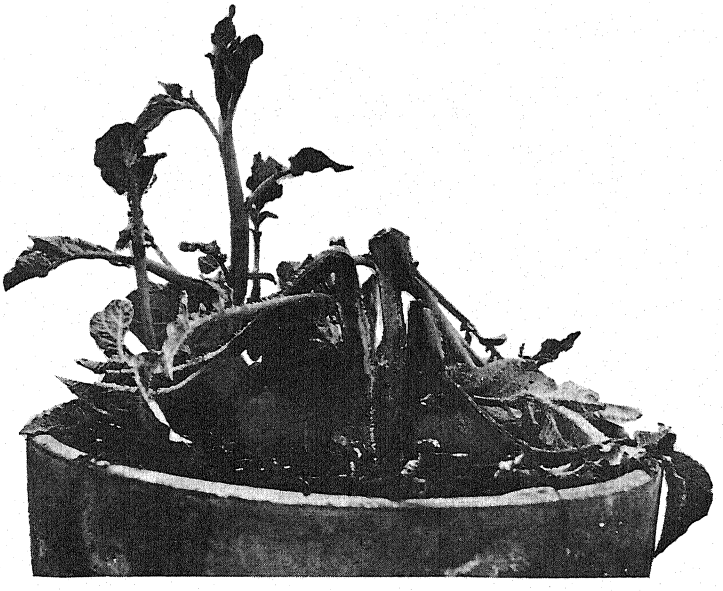

FIG. 10. Young potato plants decayed as a result of inoculating the stems with a broth culture of bacteria obtained from the tracks made by freshly hatched maggots on sterile agar.

THE RELATION OF THE INSECT TO THE BLACKLEG DISEASE

Pathogenic bacteria commonly present on eggs

Although the close association of the seed-corn maggot with potato blackleg is highly suggestive, it does not in itself prove that the insect is an agent of dissemination of the disease. In order to determine whether the insect actually spreads the pathogene, a number of eggs were collected and placed upon sterile agar, or on sterile glass near sterile agar. Upon hatching, the maggots crawled rapidly about over the agar, leaving minute tracks where their mouth parts had penetrated the agar. Within a few hours of the passage of the maggot, an examination showed colonies of bacteria developing along the tracks (Plate XV, i). In general, each colony coincided with a puncture of the agar by the mouth parts of the maggot. Broth cultures made from these colonies, when inoculated into tubers and

on young potato plants, were pathogenic (Fig. 10).[4] After this experiment had been repeated several times, with similar results in the majority of cases, it was evident that the eggs were commonly contaminated with pathogenic bacteria.

FIG. 11. Potato tubers and a carrot slice six days after inoculating with a broth culture of bacteria obtained by aseptic dissection of the abdominal contents of adult flies caught on May 5, 1925.

PATHOGENIC BACTERIA PRESENT IN BODY OF ADULT FLIES

Since the eggs commonly bear pathogenic bacteria, it seemed probable that the bacteria would be present also in the body of the insect. Therefore a number of flies were caught, chloroformed, and dissected aseptically. The contents of the abdomen were carefully removed with sterile needles and placed in a tube of broth or on an agar slant. Cultures obtained in this way also proved to be pathogenic (Fig. 11).

Pathogenic bacteria were obtained also from the excrement of flies deposited on the inside of a sterile culture tube. These preliminary experiments were performed during July, 1924. They were repeated on a larger scale during the first week in May, 1925, using flies of the first brood to emerge in the spring, and obtained before potato planting had been started. The results are given in tables 1 and 2.

PASSAGE OF BACTERIA THROUGH PUPAL STAGE

The constant association of pathogenic bacteria with the various stages of the insect suggested the possibility of biologic transmission, with the

[4] Further study of these cultures showed that they were nearly always mixed cultures of at least three species of bacteria. During the course of the work, a considerable number of apparently different species, or perhaps strains, of bacteria have been isolated from the insect or from plants attacked by the insect. No attempt has yet been made definitely to identify them, it being considered sufficient, for the present, to determine whether they were pathogenic.

TABLE 1.—*Results of aseptically dissecting adult seed-corn-maggot flies, and culturing the abdominal contents*

Fly No.	Sex	Culture No.	Growth[a]	Pathogenicity to potato tubers[b]
1	Female	A	–	
		B	+	–
2	Female	A	+	Not tested
		B	+	Not tested
5	Female	A	+	+
		B	+	Not tested
6	Female	A	+	+
8	Female	A	+	+
9	Female	A	+	+
		B	+	+
10	Female	A	+	+
11	Female	A	+	+
13	Female	A	+	+
3	Male	A	+	+
7	Male	A	–	
12	Male	A	+	–
15	Male	A	+	Not tested
16	Male	A	–	
17	Male	A	+	+

[a] –, no growth
+, growth
[b] –, not pathogenic
+, pathogenic

TABLE 2.—*Pathogenicity of bacterial cultures from excrement of flies deposited on sides of sterile culture tubes*

Fly No.	Sex	Growth	Pathogenicity
1	Female	+	Not tested
2	Female	+	+
4	Female	+	+
6	Female	+	+
7	Female	+	–
3	Male	–	
5	Male	+	+

bacteria a constant symbiont with the insect. A number of such symbiotic relationships between insects and microorganisms have been reported in recent years.

Guyénot (10), in 1907, studied the relationship between *Micrococcus flavus liquefaciens* (Flugge) and *Phormia regina* Meigen (a scavenger),

and concluded that there was a true symbiotic relationship between the two organisms. The bacteria, which were always found with the larvae, were shown to be the agents of liquefaction of the food material of the larvae, while the larvae aided the development of the bacteria by the action of their hook-like mouth parts.

Baumberger (2) has shown a similar relationship between *Drosophila* and yeast, but in this case the yeast serves as a food for the larvae and not merely as an agent of liquefaction. He thinks, too, that the larvae studied by Guyénot may also feed upon the bacteria. He concludes that "The experiments and considerations all tend to establish the principle that insects inhabiting fermenting and decaying substrata of low protein content, usually feed upon the microorganisms present and thus benefit by the power of the fungi to extract, absorb, and synthesize many non-protein nitrogenous compounds." Delacourt and Guyénot (6) and Guyénot (11) also obtained results with *Drosophila* similar to those obtained by Baumberger.

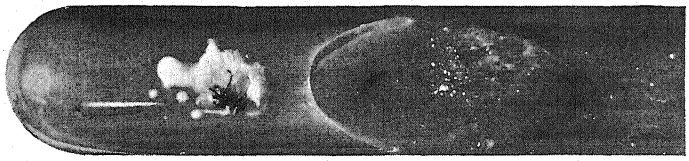

FIG. 12. Bacteria developing from an adult fly 48 hours after emerging from a surface sterile puparium. The fly became wedged between the agar and the side of the tube soon after emerging. Bacteria developed only where the fly had touched the agar. The agar immediately around the pupal skin remained sterile.

Baumberger was able to obtain sterile adults of *Drosophila* by surface sterilization of the puparia with fifty per cent alcohol, indicating that the yeast did not persist inside of the puparia. He was unable, however, to sterilize the pupae of *Musca domestica.*

Bacot (1), working with *Musca domestica,* concludes:

"1. Pupae and imagines of *Musca domestica* bred from larvae infected with *B. pyocyaneus* under conditions which exclude the chance of re-infection in the pupal or imaginal period undoubtedly remain infected with the bacillus.

"2. In the imago the infection is maximal at emergence and then diminishes suddenly.

"3. The possibility of a dangerous pathogenic microorganism being taken up by the larva and subsequently distributed by the fly is one which deserves serious consideration."

The same author, in a footnote, announces the isolation of *B. typhosus* from the interior of pupae, the larvae of which had been fed on the organ-

ism. Graham-Smith (9) obtained cultures of *B. anthracis* from 26 of 51 flies reared from larvae which had fed on meat infected with the organism.

Buchner (3), and Hertig and Wolbach (15), have described several cases of symbiosis between insects and microorganisms, in some of which the microorganisms always were found associated with the insect in all of its stages, including the egg.

In order to determine whether the bacteria associated with the seed-corn maggot could remain alive within the puparium, twelve puparia were surface sterilized in July, 1924, in the following manner: they were first soaked in 5 per cent alcohol for 5 minutes; then in an aqueous solution of mercuric bichloride (1–1000) for 3 minutes; then rinsed in 50 per cent alcohol for about 1 minute, and placed on a plate of soft beef extract agar. After 48 hours bacterial growth had developed around two of the puparia. After 72 hours the remaining ten puparia were removed, and each was placed in a separate tube of sterile beef-extract broth. After 24 hours the broth in every tube was still clear, and remained so until discarded several days later. The puparia were then transferred to new tubes of sterile broth. these also remained clear for 24 hours. The puparia were then crushed with sterile forceps. In every tube the broth became cloudy with bacterial growth within 12 hours. Transfer cultures from two of these tubes were tested and, although evidently mixed, proved pathogenic to tubers.

This experiment shows that the bacteria may remain alive within the puparia, but does not prove that they remain alive until the adult emerges. Therefore, in July, 1925, when another supply of puparia was available, this point was tested. Forty puparia were surface disinfected in the following manner. They were soaked in 50 per cent alcohol for 3 to 5 minutes, in a mercuric bichloride solution (1–1000) for 3 to 5 minutes, and again in 50 per cent alcohol for 3 to 5 minutes. Each puparium was then rinsed in a tube of sterile broth for 5 to 10 minutes. They were then removed from the broth with a sterile needle and, without rinsing, placed

TABLE 3.—*Results of experiments to determine the presence of pathogenic bacteria in adult flies emerging from surface sterilized puparia*

No. of puparia disinfected	No. proved surface sterile	No. adults emerging	No. tubes showing bacterial growth 24 hours after emergence of fly	No. cultures pathogenic
40	28	25[a]	25	9 out of 12 tested

[a] The three remaining puparia were crushed on sterile agar, and abundant bacterial growth developed from each puparium in twenty-four hours.

on agar slants in culture tubes, where they were allowed to remain until the adult flies emerged. The results are given in table 3, and illustrated in figure 12.

This experiment seems to show conclusively that the bacteria remain alive within the puparium and emerge with the adult fly in an active and virulent state.

BACTERIA NOT INSIDE OF THE EGGS

In several cases [Buchner (3), and Hertig and Wolbach (15)], micro-organisms living symbiotically with insects have been shown to live inside of the egg. It seemed desirable to determine if this were true in this case. It was decided, therefore, to attempt to surface sterilize a number of eggs. Much to the surprise of the writer, it was found that this could be done very

FIG. 13. A culture tube of sterile agar showing tracks made by a sterile maggot hatched from a surface sterilized egg. Photographed by reflected light one week after maggot emerged from the egg.

easily without injury to the viability of the egg. The uninjured shell of the eggs is very impermeable to an aqueous solution of mercuric bichloride. It was found that the eggs could be soaked in such a solution (1–1000) for as long as two hours without injury to the embryo. Almost one hundred per cent surface sterility was obtained in this way. The surface sterilized eggs, after being rinsed in sterile broth, were placed on agar slants. The maggots which hatched from such surface sterilized eggs invariably were sterile, showing conclusively that the bacteria are not carried within the eggs. The results of a number of these treatments are given in table 4. Figure 13 shows an agar slant marked with tracks of a sterile maggot photographed one week after the maggot had emerged from the egg. The agar was absolutely sterile.

The above results were confirmed by aseptic dissection of the eggs from the bodies of female flies. The eggs obtained in this way were placed separately on the surface of sterile agar. Those remaining free from bacterial growth after seven days were removed and crushed in a tube of sterile broth or on an agar slant. In every case the broth remained sterile.

PROBABLE METHOD OF SURFACE CONTAMINATION OF EGGS

The results presented in table 4 show that the bacteria are not carried within the egg. The question of how the eggs become contaminated nat-

TABLE 4.—*Results of experiments to determine the presence of bacteria inside of eggs of the seed-corn maggot*

Egg no.	Treatment	Time	Surface sterility	Viability	Maggot sterility
1	Mercuric bichloride 1–1000	1 min.	Sterile	Viable	Sterile
2		2 min.	do	do	do
3		3 min.	Not sterile	do	Not sterile
4		15 min.	Sterile	do	Sterile
5		do	do	do	do
6		do	do	do	do
7		do	do	do	do
8		do	do	do	do
9		do	do	do	do
10		do	do	do	do
11		do	do	do	do
12		do	do	do	do
13		do	Not sterile	do	Not sterile
14		1 hr. and 45 min.	Sterile	do	Sterile
15		do	do	do	do
16		do	do	do	do
17		do	do	do	do
18		do	do	do	do
19		do	do	Not viable	
20	Hot formaldehyde 1 pt. to 15 gallons	2 min.	Not sterile	Viable	Not sterile
21		do	do	do	do
22		do	do	Not viable	
23		do	Sterile?	Viable	Not sterile
24		do	do?	do	do
25		do	Sterile	Not viable	
26		do	do	do	
27		do	do	do	
28		do	do	do	
29		do	do	do	
30		do	do	do	
31[a]		do	do	do	

[a] Many more were later surface sterilized in $HgCl_2$ 1–15, a record of which was not kept, but all surface sterile eggs which hatched gave rise to sterile maggots.

urally arises. A study of the anatomy of the adult female fly shows how this very probably takes place. The end of the ovipositor and anal tract are shown in Plate XV, e. The openings through which the excrement and the eggs pass are in close proximity, and are surrounded by bristly segments. The eggs are moist and covered with a gelatinous substance when deposited, and, since the excrement of the fly contains the bacteria, it would appear very difficult for the eggs to be deposited without becoming contaminated. Maggots in the act of hatching have been observed, and they always crawl

over the old shell for a few moments after emerging. It is very probable that the maggot gets the bacteria into its system at this time.

BACTERIA NECESSARY FOR DEVELOPMENT OF LARVAE

Baumberger (2) has shown that *Drosophila* living in fermenting fruit are dependent on yeast cells for their food supply and that sterile larvae are unable to grow normally on sterile fruit. They grow well, however, with the addition of living yeast. In the experiment reported above, in which sterile larvae of the seed-corn maggot were obtained by surface sterilizing the eggs, it was observed that the few which became contaminated with bacteria grew and increased in size rapidly, although they did not pupate normally; while those remaining sterile did not increase appreciably in size, although they remained alive for more than a week. Bacteria were added to a few of these after they had remained about five days on sterile agar. Within forty-eight hours they had more than doubled in size. Since these maggots were growing on slants of beef-extract agar, it was decided to repeat the experiment, using sterile and non-sterile potato plugs as media. A quantity of eggs was obtained, surface sterilized, and placed on sterile agar. After the maggots emerged, they were allowed to remain on the sterile agar for twenty-four hours, to determine whether they were sterile. Forty-eight sterile maggots were obtained in this way. As soon as proof of sterility was obtained, they were removed to sterile media, as indicated in table 5. One half of the tubes were inoculated with pathogenic bacteria; the rest were allowed to remain sterile. The results are given in table 5, and illustrated in Plate XV, k. They show clearly that the presence of bacteria is necessary for the normal growth of the larvae when feeding on potato tubers. They indicate, however, that the potato furnishes something essential to normal growth, since the larvae growing on inoculated beef extract agar did not pupate and emerge normally.

THE PRODUCTION OF THE DISEASE BY "INOCULATING" SEED PIECES WITH EGGS OF THE SEED-CORN MAGGOT

In the spring of 1925, attempts were made to produce the disease by placing eggs in the soil near the seed pieces. Eggs were collected from the soil near seed pieces which were exposed on the surface of the ground. A total of 900 seed pieces were "inoculated." Holes about four inches deep were dug in the soil, and a freshly cut seed piece was put in each hole. From 3 to 5 eggs were placed in the soil near each seed piece and covered with about four inches of loose soil. Nine rows of 100 "inoculated" seed pieces and 50 "uninoculated" seed pieces were planted. Notes were taken six weeks after planting. Of the 900 "inoculated" plants, 46, or a little

TABLE 5.—*Results of experiments to determine the relation of bacteria to the development of the larvae of the seed-corn maggot*

Sterile maggot No.	Medium	Result	Sterile maggot No.	Medium	Result
1	Sterile potato plug	Very little growth, maggot remaining transparent and dying after 10 to 20 days	1a	Inoculated potato plug	Normal growth; pupated and emerged
2	do		2a	do	do do
3	do		3a	do	do do
4	do		4a	do	do do
5	do		5a	do	do do
6	do		6a	do	Pupated early; did not emerge[a]
7	do		7a	do	Dead before pupating; good growth
8	Sterile beef extract agar		8a	Inoculated beef extract agar	Grew slowly; pupated early
9	do		9a	do	do do
10	do		10a	do	do died before pupation
11	do		11a	do	do do
12	do		12a	do	do do
13	do		13a	do	do do
14	do		14a	Inoculated potato plug	Normal growth; pupated and emerged
15	Sterile potato plug		15a	do	do do
16	do		16a	do	do do
17	do		17a	do	do do
18	do		18a	do	do do
19	do		19a	do	do do
20	do		20a	do	do do
21	do		21a	do	do do
22	do		22a	do	do do
23	do		23a	do	do do
24	do	Normal growth; pupated and emerged[b]	24a	do	do do

[a] Some of the potato plugs with maggots and bacteria were to moist for good growth, probably explaining the action of those which did not pupate and emerge normally.

[b] Accidental contamination.

more than 5 per cent, developed typical blackleg, while there was not a single blackleg plant in the checks. There were also 45 skips in the "inoculated" rows against 4 skips in the checks. The results, although not very striking, show that blackleg may be produced by "inoculating" the seed pieces with eggs of the seed-corn maggot.

The writer is convinced that, with a different method of "inoculation" and more favorable weather conditions, a much higher percentage of diseased plants can be obtained. At the time this experiment was started, it was believed that in nature many of the eggs were deposited on the seed pieces before planting. If this were true, the method would have been somewhat comparable. However, later observations showed that in all probability most of the eggs are deposited after planting and much nearer the surface of the soil, or at least in open cracks. In the methods used, the eggs were under about four inches of soil which was packed tightly by heavy rains[5] shortly after planting.

POSSIBILITIES OF CONTROL

The problem of control is a difficult one, and no definite recommendations can be given at this time. Since it has been found that most of the eggs are deposited in the soil after planting, it is evident that protection of the seed pieces before planting is not sufficient. That the eggs are very resistant to mercuric bichloride and hot formaldehyde is shown by the data given in table 4. However, the larvae of closely related insects are known to be very susceptible to mercuric bichloride. Some preliminary experiments indicate that seed treated after cutting with a solution of mercuric bichloride 1–1000 and allowed to dry retain sufficient mercury to kill maggots which attack it. Seed pieces treated with hot formaldehyde were readily attacked by the maggots and decayed rapidly. That the latter treatment was in no way injurious to the maggots was indicated by the fact that they reached normal size, pupated, and emerged as adult flies. This may explain the fact that mercuric bichloride treatments in many cases have given better control of blackleg than formaldehyde treatments. More experiments are necessary, however, before any definite conclusions can be reached.

DISCUSSION

The observations and experiments reported above show conclusively that plant pathogenic bacteria are constantly associated with the seed-corn mag-

[5] The rainfall at this station was extremely heavy during the five weeks immediately following the planting of the potatoes in this experiment. The official government Weather Bureau station located at the Northwest School and Station, Crookston, reported rain on 27 days between May 18, the first date of planting, and June 30. A rainfall of 3.96 inches fell on May 23.

got in the various stages of its development, and that potato blackleg[6] may develop following the attack of the larvae on otherwise healthy seed pieces. From the standpoint of the development of blackleg, the insect acts not only as an agent of dissemination but as an agent of inoculation. It has been shown that, although seed pieces may be partly decayed by the blackleg pathogene, they may be able to cork off the decay and produce a healthy plant if planted under reasonably good growing conditions. On the other hand, if a seed piece is attacked by a seed-corn maggot, it is unable to check the decay. Wound cork formation is not sufficient to overcome the combined action of the maggot and pathogenic bacteria. Any cork that is formed is soon destroyed by the maggot, which at the same time hastens the development of decay by spreading the bacteria throughout the tissues of the tuber.

It should be pointed out here that the experiments reported in this paper dealing with artificial inoculation have no bearing on natural infection of tubers through the stolons of diseased plants. This means of infection constitutes an entirely different phenomenon, which will be discussed in a subsequent paper.

It also should be understood that dissemination by the seed-corn maggot is not considered to be the only means of dissemination of blackleg. Moreover, the writer is of the opinion that in many cases maggots may be found in the seed pieces of plants which would have produced blackleg in the absence of the insect. In fact, the possibility that the insect attacks only decaying plants has been suggested (28), and was seriously considered, but the observations on the egg-laying habits and the decaying action of young maggots when placed on perfectly sound seed pieces, together with the constant association of pathogenic bacteria with the insect, convinced the writer that the maggots could, and commonly do, attack perfectly healthy seed pieces and inoculate the plants with blackleg. In Minnesota the presence of this insect will explain many, but probably not all, of the outbreaks of blackleg in fields planted with seed known to be free from blackleg infection.

The possibility of infection from this source should always be taken into consideration in field experiments on blackleg. It is very likely that many cases of blackleg infections which, in past experiments, have been interpreted as seed piece dissemination may have been due to attacks of the seed-corn maggot. It also must be considered in judging the results of seed potato certification.

In view of the changes that are known to take place in the puparium prior to the emergence of the adult, it was very surprising to find that the

[6] The term "blackleg," as used here, refers to a bacterial necrosis of the stems of potato plants answering the general descriptions of that disease as described in the literature, without implying that the condition is caused by any particular species of bacteria.

bacteria passed through this stage and emerged with the adult fly unharmed. It would be interesting to follow the bacteria through this stage by histological methods. Although material has been fixed and imbedded, this has not yet been done.

The discovery of the relation of this insect to potato blackleg constitutes, insofar as the author knows, a distinctly new type of insect transmission of a bacterial plant disease. It is biological transmission in the sense used by Rand and Pierce (26), although in animal pathology the term "biological" generally is used only when the insect constitutes the only known means of transmission.

The facts are very suggestive as to the relation of other similar insects to bacterial and possibly fungous diseases of plants. It may be of interest to mention that most of the data presented here for the seed-corn maggot and potato blackleg have been found to hold true for the cabbage maggot and the soft rot which usually accompanies its attack.

The relationship between the insect and the bacteria should, in all probability, be classed as symbiotic. It has been shown that the bacteria are not only beneficial to, but necessary for, the development of the larvae. Likewise, it is obvious that the insect contributes to the welfare of the bacteria by disseminating them, by inoculating them into the plant, by aiding them in destroying the plant tissue, and by providing them with a sure means of hibernation.

The relationship is of interest in considering the origin of the parasitic habits of this type of insect. Frost (7), in his monograph on the leaf mining diptera of North America, states: "It is possible that the scavenger habit developed before the plant-feeding habit, the larvae having first gained entrance to the plant through decayed roots and stems. Some writers would take exception to this hypothesis, contending that insects first became established on living material and, as the host died, became adapted to a saprophytic, or scavenger habit. It seems plausible, however, that in the leaf-miners the adaption is working in the reverse direction, and that the scavenger habit is the more primitive. According to this hypothesis, the larvae, having first entered the plant as scavengers, became adapted to an existence within the living host and finally migrated to the stems and leaves where they are now known as leaf-miners." Following this same line of thought, it would seem probable that, during the evolution of the insects, certain scavengers developed the habit of depositing their eggs in plants attacked by certain plant pathogenic bacteria until these organisms were included among their internal symbionts. With plant pathogenic bacteria as symbionts, maggots from eggs deposited on healthy plants would be able to induce the decay and thus survive. In this way the parasitic habit may

have arisen. On the other hand, when we consider that the association with the bacteria enables the insect to shorten its life cycle (2), it may be argued that this is the most highly developed state. The writer must confess that he does not have enough information about the habits of other species of insects on which to base an opinion. Attention is called to the possibilities because of their evident bearing on a very interesting problem. More extensive investigation of the food habits of such insects is desirable.

SUMMARY

1. Investigations of numerous outbreaks of potato blackleg in Minnesota showed that much of the disease could not be accounted for by seed piece transmission.

2. Attempts to produce the disease by inoculating the seed pieces were unsuccessful because of the striking ability of the sprouting seed piece to cork off the decay. These results indicate that contamination of the seed piece at the time of cutting usually is not an important factor in the dissemination of the pathogene.

3. Observations in Minnesota extending over three years revealed a close association between the seed-corn maggot (*Phorbia fusciceps* Zett.) and potato blackleg.

4. The seed-corn maggot is known to be parasitic on a wide range of host plants, including potatoes; and a soft rot of attacked tissues characteristically follows its injury.

5. Eggs of the insect are deposited on, or in, the soil near healthy seed pieces or sprouts.

6. The eggs are commonly surface contaminated with plant pathogenic bacteria.

7. The larvae inoculate the seed pieces with the bacteria and aid the development of the disease by destroying or preventing the formation of wound cork.

8. The bacteria are constantly associated with the insect, passing through the pupal stage, and emerging with the adult fly in a virulent state. Pathogenic bacteria are commonly present in the intestinal tract of adult flies.

9. The bacteria do not occur inside of the eggs. The surface of the eggs probably becomes contaminated at time of deposition, when, coated with a sticky fluid, they pass between the anal appendages.

10. Sterile maggots, obtained from surface sterilized eggs, were not able to grow on sterile potato tubers but grew normally when bacteria were added, showing that the bacteria are essential for the development of the maggots.

11. The maggots developed slightly better on inoculated potato plugs than on inoculated beef-extract agar.

12. Typical blackleg was produced by "inoculating" healthy seed pieces with eggs of the seed-corn maggot.

13. The seed-corn maggot is an agent of dissemination, inoculation, and hibernation of plant-pathogenic bacteria.

14. The association between the bacteria and the insect appears to be one of mutualistic symbiosis, and constitutes a type of insect transmission of a bacterial plant disease not previously described.

15. The facts presented here may have a bearing on the problem of the origin of parasitism among dipterous insects.

16. No measures for the control of the insect are recommended, although preliminary experiments indicate that a thin coating of mercuric bichloride over the surface of cut seed pieces may be effective in preventing injury.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY FARM, ST. PAUL.

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EXPLANATION OF PLATE XV

a. Adult female seed-corn-maggot fly. (Approx. 10 ×.)

b. Eggs of the seed-corn maggot. (Approx. 50 ×.)

c. An egg of the seed-corn maggot. (Approx. 100 ×.)

d. Adult female seed-corn-maggot fly. (Approx. 1¾ ×.)

e. Tip of ovipositor of adult fly showing the bristly segments surrounding the anal opening and the opening through which the eggs pass. Surface contamination of the eggs probably takes place here.

f. A larva of the seed-corn maggot almost fully developed. (Approx. 1½ ×.)

g. A hind leg of a male fly of the seed-corn maggot, showing the row of bristles along the middle segment which is a characteristic of this species.

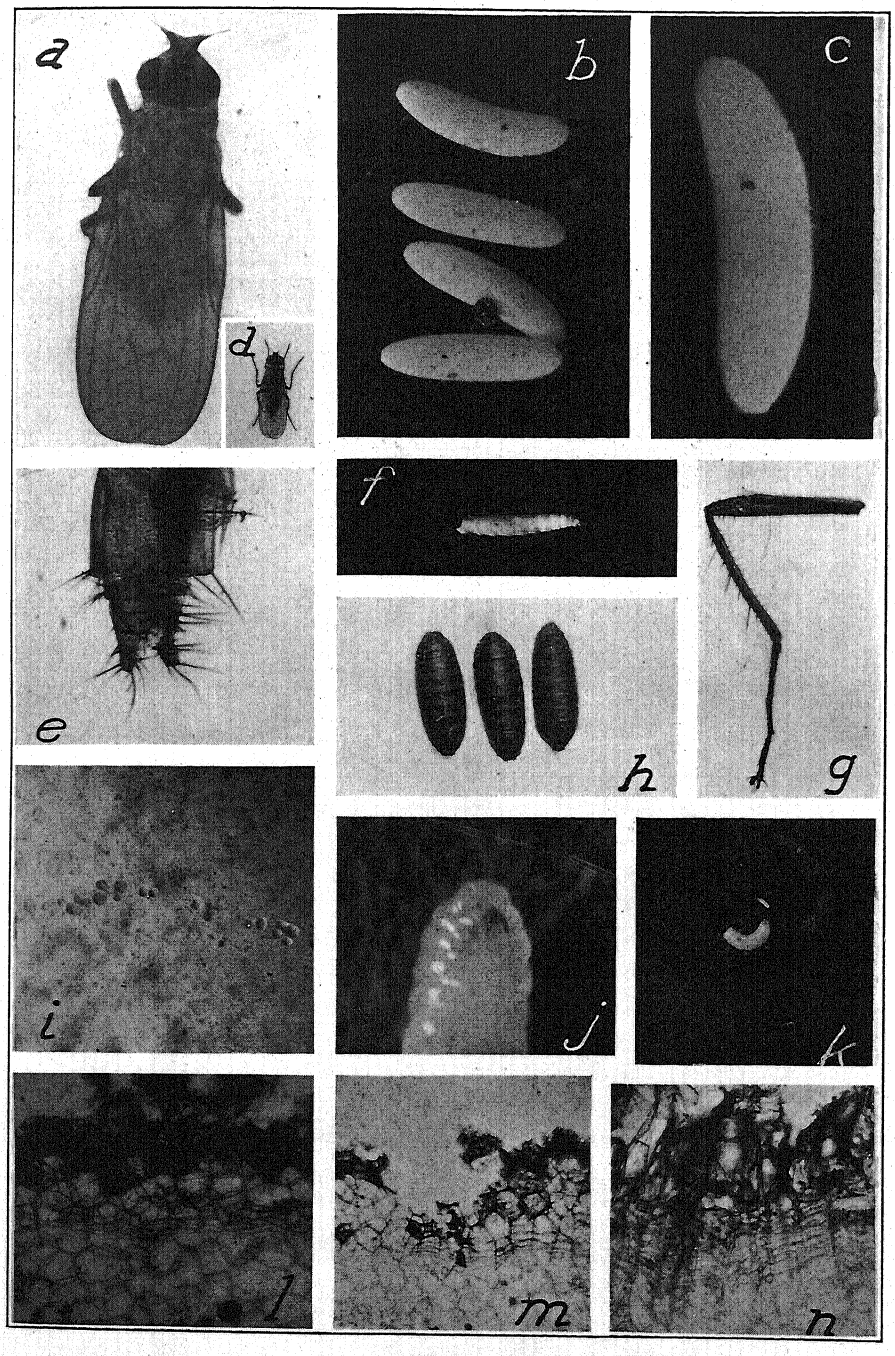
h. Three puparia of the seed-corn maggot. (Approx. 3 ×.)

i. Bacterial colonies developing in the tracks of a maggot freshly hatched on sterile agar.

j. The mouth parts of a seed-corn-maggot larva, showing the sharp claw-like structures with which it attacks and burrows into plant tissue.

k. Two larvae hatched from surface sterile eggs. The upper and small one had fed for twelve days on a sterile potato plug. The larger one fed for twelve days on a potato plug inoculated with plant pathogenic bacteria.

l, m, and n. Sections through the healed surface of inoculated seed pieces about two months after planting, showing the cork layer which has completely and effectively walled off the bacteria, thus preventing further decay.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PSEUDOMONAS (PHYTOMONAS) PISI SACKETT, THE CAUSE OF A POD SPOT OF GARDEN PEAS

C. A. LUDWIG

INTRODUCTION

Pseudomonas pisi Sackett (*Phytomonas pisi* (Sackett) S. A. B.),[1] which was described as the cause of a stem blight of field and garden peas in Colorado, has recently been found to produce a pod spot also.

When the spots are young they have the usual translucent, water-soaked appearance characteristic of bacterial lesions; but they usually become yellowish, and finally brown, as the pods mature. When the pods are ripe, both the healthy and diseased tissues have the characteristic stiff papery texture of pea pods. The brown color of the spots, however, sets them off, in strong contrast, from the lighter color of the healthy tissue (Plate XVI, 5). If young pods are infected severely, they shrivel up and die. The diseased tissues swarm with bacteria. The symptoms on the other parts of the plant have been described by Sackett[1] and in the Plant Disease Reporter,[2] and will not be listed here.

ISOLATION OF THE ORGANISM

The organism used in these studies was isolated by the usual poured plate method[3] from a specimen of spotted pods received in April, 1922, from Mr. Rudolph Farmer, then horticultural specialist on the Extension Division staff of Clemson College. They came from a planting near Latta, South Carolina; and there was no report of any stem blight accompanying or preceding the spots. The chief commercial damage was in the appearance of the pods, which affected the marketability of the crop.

INOCULATIONS

The results of the inoculations are given in table 1. Checks, similarly treated except for inoculation, remained healthy in all instances. Except as noted, the inoculations were made in the greenhouse.

[1] Sackett, W. G. A bacterial stem blight of field and garden peas. Col. Agr. Exp. Sta. Bul. 218. 1916.

[2] U. S. Dept. Agr. Plant Disease Reporter 8: 13–14. 1924.

[3] These plates were poured by Mr. W. B. Aull, associate professor of bacteriology. Assistance in this work from the following is acknowledged also: Mrs. W. B. Aull, Mr. F. O. Myers, and Mr. J. H. Hunter.

Inspection of table 1 will show that most of the inoculation work for the first three years has been grouped by seasons and recorded in general terms instead of taking each inoculation separately. This is to be regretted, but it is necessary, owing to a fire which burned the Agricultural Hall at Clemson College on April 2, 1925, and destroyed most of the notes concerning the organism which had been accumulated up to that time. Most of the early work must therefore be reported from memory and can not be given in detail.

The records show that there is more or less variation in susceptibility among different varieties of peas. The variety Telephone was by far the most susceptible of the three or four varieties used. It was observed also that young, succulent, rapidly growing tissues are more easily infected than tougher, more mature ones. The Telephone variety is rank in its habit of growth and its tissues are very tender and succulent when young. It is possible that the greater susceptibility of this variety observed here may be ascribed to this condition.

The circumstances, in the order of decreasing importance, which favored infection were wounds, rapidly growing succulent tissues, and high humidity (secured by moist chambers). The moist chambers were quite unimportant. In fact, it was found so easy to produce infections without them that most of the later work was done without coverings of any kind.

The presence of wounds was very important, however. There were some infections where no known wounds were present, but these were very few as compared with the number which developed following injuries. It is difficult to believe that an organism exhibiting the infection characteristics of this one could produce a severe epidemic in the absence of some wholesale source of mechanical injuries to the crop affected. In the inoculations carried out in 1924, much of the work was done in the writer's garden, and no accidental or natural infections were discovered. It is worthy of note in this connection that Sackett emphasized the importance of mechanical injuries as predisposing to the disease, even with a strain of bacteria capable of producing stomatal infections in great abundance.

The fact that the one seed inoculation test which we have been able to make failed to produce the disease would indicate that the bacteria are probably not seed-borne. It is recognized, of course, that one such test is insufficient to be considered as proof of the point.

The only plants other than garden peas which were inoculated were red and white clover. There was not the slightest evidence of infection in either case.

CULTURAL CHARACTERISTICS AND IDENTITY OF THE ORGANISM

The organism was studied on the following media: nutrient agar, nutrient gelatin, milk, litmus milk, potato plugs, nutrient broth, nitrate broth,

peptone broth, starch agar, dextrose broth, lactose broth, saccharose broth, and glycerin broth.

The behavior of the organism on these media, with exceptions noted below, was similar to that of Sackett's *Pseudomonas pisi*. Accordingly, an attempt was made to secure an authentic culture of that organism for purposes of comparison. Professor Sackett's type culture had been lost, however, and no other could be obtained. Under these circumstances Professor Sackett kindly examined the South Carolina culture, made the necessary tests, and reported that, in his opinion, the organism is the same as the species he described, although possibly a different strain. The most important difference he noted was the failure of the South Carolina strain to produce a curd in milk, while his produced a soft curd. He did not observe reduction of nitrate, although our tests indicate a weak action of this kind. In litmus milk he noted more marked color banding than was the case with the Colorado organism, but he does not consider this an important difference. Both organisms exhibited considerable variation in this regard. Our results indicate that the organism does not digest the casein, at least not quickly, as the addition of acid some months after inoculation and after the culture had become thoroughly blue threw down a curd, in addition to reddening the litmus.

A further difference in the two strains lies in the ease of securing infections. Sackett's strain produced stomatal infections with great ease. No such infections with the South Carolina organism have been noted.

All things considered, it seems best, at least for the present, to consider the organism as a strain of *Pseudomonas pisi* Sackett (*Phytomonas pisi* (Sackett) S. A. B.).[4] A culture is being deposited with the type culture collection at the McCormick Institute, Chicago, Illinois.

LONGEVITY OF THE ORGANISM IN CULTURE

During the summer of 1923 it became impossible to obtain transfers of the original culture 4; and the practice was adopted of transferring the remaining cultures—A, B, C, and D (see table 1)—at intervals of about two weeks. For more than a year the old cultures were not discarded, so that at the end of that time a large number were on hand, many of which had been thoroughly dried for months. They were carried on ordinary nutrient agar.

Late in June or early in July, 1924 (record of exact date burned), sterile nutrient broth was poured into each tube, and subcultures were made from those which clouded. The tubes so treated contained various transfers of

[4] Society of American Bacteriologists. Manual of determinative bacteriology. p. 181. Williams and Wilkins Co. 1923.

TABLE 1.—*The results of inoculating several varieties of garden peas and two species of Trifolium with Pseudomonas pisi Sackett in 1922–1925.*

Date	Test plant[1]	Parts inoculated	Source of inoculum	Manner of inoculation	Result	Notes
1922[2]	Garden pea	Pods and stems	Original isolations	Needle pricks; moist chambers used	−	
1923[2]	Garden pea, Tel. included	Pods	Original culture 4, some of the reisolations	Needle pricks; moist chambers used	+	Several infections, 4 reisolated strains, A, B, C, and D
1924[2]	Garden pea, about three varieties, including Tel.[3] Tel.[4]	All parts	Cultures A, B, C, and D	Spores rubbed or sprayed on uninjured plants and on plants pricked with a needle; with and without moist chambers	+	Where infections occurred without pricking there were possibly other minute wounds
1924[2]	Tel., L.E., E.E.A.		Culture B2Y (reisolation of same season)		+	
March, 1925[2]	Tel., L.E., E.E.A.	Stems	Culture 4	Needle pricks; moist chambers used	−	
March, 1925[2]	Tel., L.E., E.E.A.	Stems	Culture B2Y	Needle pricks; moist chambers used	+	
March 26, 1925	E.E.A.	Pods	Culture 4	Needle pricks; moist chambers used	+	See plate 1
April 17, 1925	E.E.A.	Pods	Spot on pod from inoculation of March 26	Needle pricks; moist chambers used	+	See plate 2
April 24, 1925	E.E.A.	All parts	Spot on pod from inoculation of April 17	Needle pricks; no moist chambers used	+	Leaves not affected
April 24, 1925	E.E.A.	Pods	Spot on pod from inoculation of April 17	Inoculum rubbed gently on pod	+	

TABLE 1.—*Continued.*

Date	Test plant[1]	Parts inoculated	Source of inoculation	Manner of inoculation	Result	Notes
April 29, 1925	E.E.A., Tel.	Stems	Spot on pod from inoculation of April 24	Needle pricks; no moist chambers used	+	More vigorous infection on young succulent parts and on Tel.
May 7, 1925	L.E., Tel.	All parts	Spot on pod from inoculation of April 24	Inoculum sprayed on uninjured plants; no moist chamber used	+	See plate 4
May 7, 1925	L.E., Tel.	Pods and stems	Spot on pod from inoculation of April 24	Needle pricks and spray; no moist chamber used	L.E. – Tel. +	See plate 5
May 7, 1925	L.E., Tel.	Leaves	Spot on pod from inoculation of April 24	Needle pricks and spray; no moist chamber used	L.E. – Tel. +	See plate 3
Feb.[2] or March, 1925	Tel., E.E.A., L.E.	Seeds	Culture 4 or B2Y	Seed soaked 15 minutes in bacterial suspension	–	
	Trifolium pratense L.	Stems and leaves		Needle pricks; moist chambers used	–	
	Trifolium hybridum L.	Stems and leaves		Needle pricks; moist chambers used	–	

[1] Varietal names of garden peas are abbreviated as follows: Tel. = Telephone; L.E. = Lightning Excelsior; E.E.A. = Extra Early Alaska.

[2] Records burned in the fire of April 2, 1925.

[3] Many of these inoculations were made in the writer's garden.

[4] Inoculation made in the writer's garden.

cultures A, B, C, and D, and the last few transfers of culture 4. The subcultures from the oldest ones which grew were put through physiological tests in comparison with the current transfers of A, B, C, and D. The earliest dated transfers, from which cultures were secured, which agreed in physiological tests with the regular cultures, were as follows:

June 1, 1923	4 and A	June 18, 1923	B, C, and 4
June 8, 1923	B	June 22, 1923	A and B
June 9, 1923	4		

Culture 4, recovered from transfer of June 1, 1923, was later (1925. See table 1) inoculated into pea pods and produced typical infection. This culture, therefore, was still viable and virulent after being dried out for several months and without transfer for over a year. Its total continuous life on artificial media was approximately three years.

DISTRIBUTION AND SERIOUSNESS OF THE DISEASE

The first record of this disease in South Carolina seems to have been the one from Latta in 1922. At that time it appeared to be merely an unimportant pod spot. Since then, however, it has taken the form of a stem blight which reduces the yield seriously. It was severe both in 1924 (see footnote 2) and in 1925.[5] It is also a serious factor in other parts of the country.

CONTROL

Two facts determined by this study have a bearing on the problem of control. First, the apparent necessity for wounds before abundant infections can take place indicates that methods of cultivation which reduce mechanical injuries to a minimum will help keep the infection down. Secondly, the difference noted in varietal susceptibility suggests that varieties which are sufficiently resistant to suffer little damage from the disease can be found or developed. The latter is probably the most promising line of attack on the control problem. This opinion is shared with Sackett, who based his conclusion on field observations of the susceptibility of several varieties of field peas.

SUMMARY

A pod spot of peas, discovered near Latta, South Carolina, in 1922, is caused by an organism believed to be a strain of *Pseudomonas pisi* Sackett. This bacterium, as shown by these studies, is an active wound parasite in pods, stems, and leaves of the garden pea, particularly when the tissues are young and succulent. Occasional infections without known wounds occur.

[5] Personal communication by Dr. W. D. Moore, assistant plant pathologist in extension work, Clemson College, S. C.

Under some circumstances it is able to live in culture without transfer for over a year, even without protection from drying. Some varieties of peas seem to be more resistant than others, and the most promising line of attack on the problem of control appears to be an attempt to find, or develop, suitable resistant or immune varieties.

SOUTH CAROLINA AGRICULTURAL EXPERIMENT STATION,
CLEMSON COLLEGE, SOUTH CAROLINA.

EXPLANATION OF PLATE XVI

1. Pod of Extra Early Alaska garden pea inoculated with culture 4. Inoculated March 29, 1925; photographed April 7.

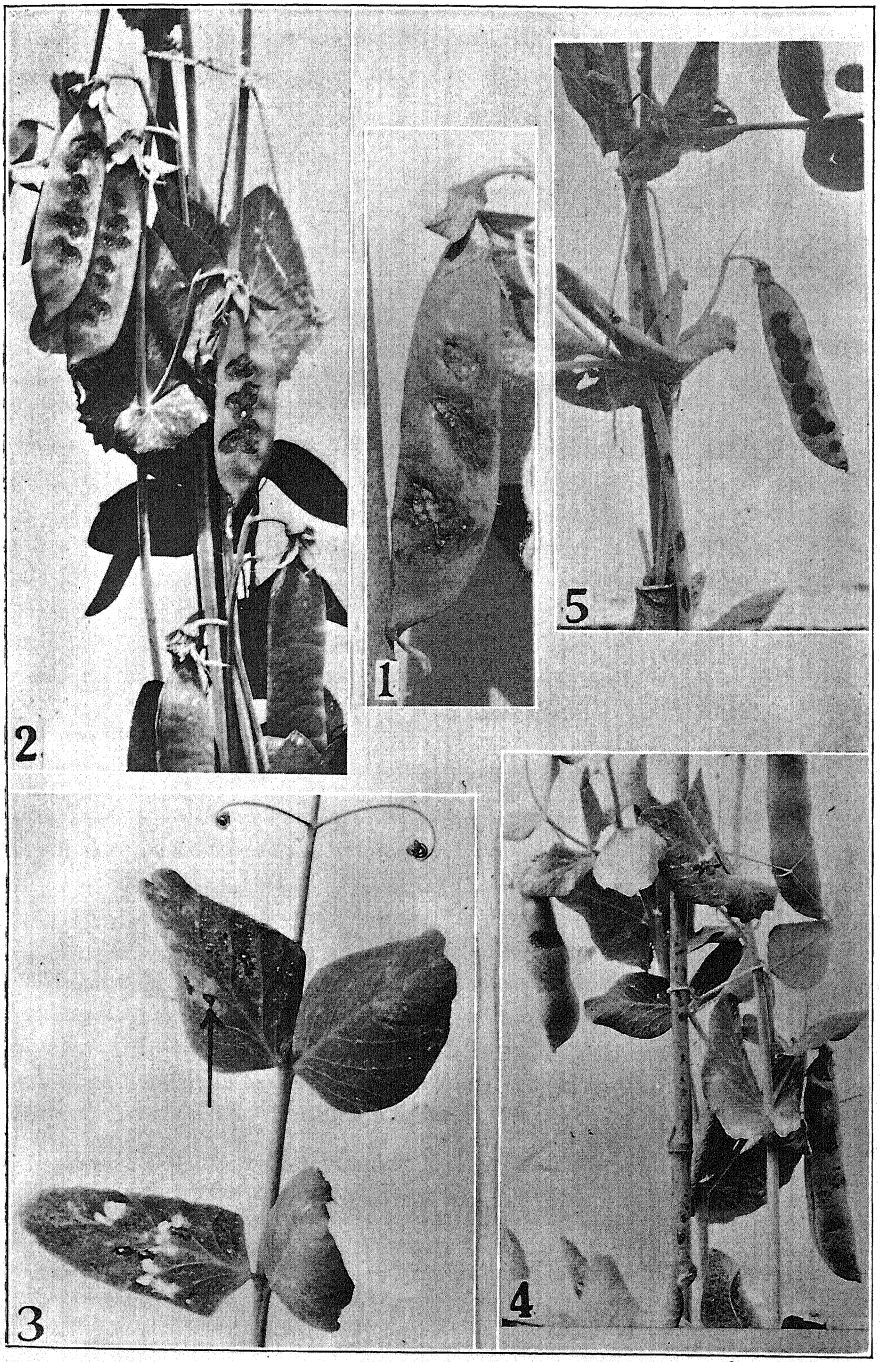
2. Pods of Extra Early Alaska pea inoculated April 17, 1925, from one of the spots on the pod shown in fig. 1. Photographed April 23.

3. Lesions on leaf of Telephone pea. Inoculated May 7, 1925; photographed June 1. The arrow points to one of the lesions.

4. Spots on pods of Telephone pea produced by spraying the inoculum onto the plants without wounding. Inoculated May 7, 1925; photographed May 19.

5. Stem and pod lesions on Telephone pea produced by spraying the inoculum onto the wounded plants. Inoculated May 7, 1925; photographed June 1. The pod in this case is nearly ripe.

Note: There was a severe infestation of red spider in the greenhouse while the inculation experiments were in progress, and the grayish appearance of the leaves in the figures is due to injury from this source.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FACTORS AFFECTING THE DEVELOPMENT OF FLAX RUST, MELAMPSORA LINI (PERS.) LEV.

HELEN HART[1]

Rust, caused by *Melampsora lini,* is very injurious to fiber flaxes, often ruining the fiber. Seed flax does not appear to be severely injured except in epidemics, which are not uncommon in the flax-growing states. Barker (24) observed that seed flax often was stunted or killed outright when young plants were heavily infected. But older plants also may be very severely injured. The writer saw plants of N. D. R. 114 from Meeker County, Minnesota, in 1925 which were quite ruined by the rust. The infection on the pedicels was so heavy that the seed was badly shriveled and the yield very low. The disease seems most destructive on low-lying soils, and is especially injurious in the Red River Valley of North Dakota and Minnesota. Brentzel (23) reported that local losses in the Red River Valley in 1919 ranged from a trace to 100 per cent. The average annual loss for North Dakota is estimated at one per cent of the entire crop. In Minnesota the disease occurs quite generally throughout the State, and causes an annual loss of something less than one per cent (24).

REVIEW OF PREVIOUS INVESTIGATIONS

In 1801, Persoon (13) described a rust collected on *Linum usitatissimum* L. and *L. catharticum* L. and named it *Uredo miniata* var. *lini.* It was not until 1847 that Léveillé (7) placed the fungus in the genus *Melampsora* under the name *Melampsora lini.* Although flax rust has been known so long, it has been but little investigated until recent years. Körnicke, in 1865, probably was the first of the earlier workers to suggest a possibility of specialization within the species. Since 1910, Palm (12) and Bucheim (4) have investigated this phase of the subject and have concluded that the rust is not the same on all species of *Linum.* Arthur (2) showed that *Melampsora lini* is eu-autoecious, but he did not find it specialized for a

[1] Cooperative investigations between the U. S. Department of Agriculture, Bureau of Plant Industry, Office of Cereal Investigations, and the Minnesota Agricultural Experiment Station. Published with the approval of the Director as paper No. 586 of the Journal Series of the Minnesota Agricultural Experiment Station.

The writer wishes to acknowledge her indebtedness to Doctor E. C. Stakman for invaluable assistance throughout the investigation.

particular species of *Linum*. The cytology and sexual fusions of *M. lini* have been described by Fromme (6), while Tobler (21) recently studied the histological effects of the pathogene upon the host tissues. In Ireland, Pethybridge and coworkers (14, 15, 16) have been investigating the general aspect of flax rust since 1920.

OBJECTS OF THE PRESENT INVESTIGATION

The investigation was undertaken to determine: (1) the factors affecting infection, (2) the effect of environmental factors on the development of rust subsequent to infection, (3) the manner of entrance of the uredinial germ tubes into susceptible and resistant hosts, (4) the histological relations between pathogene and host, (5) the possible occurrence of physiologic forms.

FACTORS AFFECTING THE GERMINATION OF SPORES

Aeciospores. Aeciospores germinate readily in distilled water, each spore sending out from one to five short, thick germ tubes. Under favorable conditions, one or two of the germ tubes develop faster than the rest, so that the growth is concentrated in one or two long infection threads. Under less favorable conditions, the germ tubes develop simultaneously and none of them become very long. Under optimum conditions, aeciospores begin to germinate within 45 minutes, and within 1½ hours they often have produced germ tubes 20 microns long.

Aeciospores do not germinate in a saturated atmosphere, but do so readily when floated on distilled water. It is well known that the spores of certain fungi germinate more readily when host tissue is added to water, some stimulatory substance apparently being supplied by the host. When crushed flax tissue was added to distilled water, the percentage of spore germination increased very slightly. Host tissue apparently does not greatly stimulate the germination of aeciospores.

Aeciospores germinate within a wide range of temperature, the minimum lying close to 0° C., while the maximum is between 26° and 27° C. The optimum is about 18° C., at which temperature the highest percentage of spores germinate and produce the most vigorous germ tubes. Those formed at the higher and lower temperatures are weaker and grow more slowly (table 1).

Urediniospores. Each germinating urediniospore sends out one or more germ tubes from its equatorial germ pores. The thin-walled, reddish-orange tubes usually are branched, and, as in the case of aeciospores, one, or sometimes two, grow faster and more vigorously than the rest (Fig. 1, C). Under optimum conditions, the small, knob-like germ tubes push out through the pores within one and one-half hours (Fig. 1, A). Under optimum con-

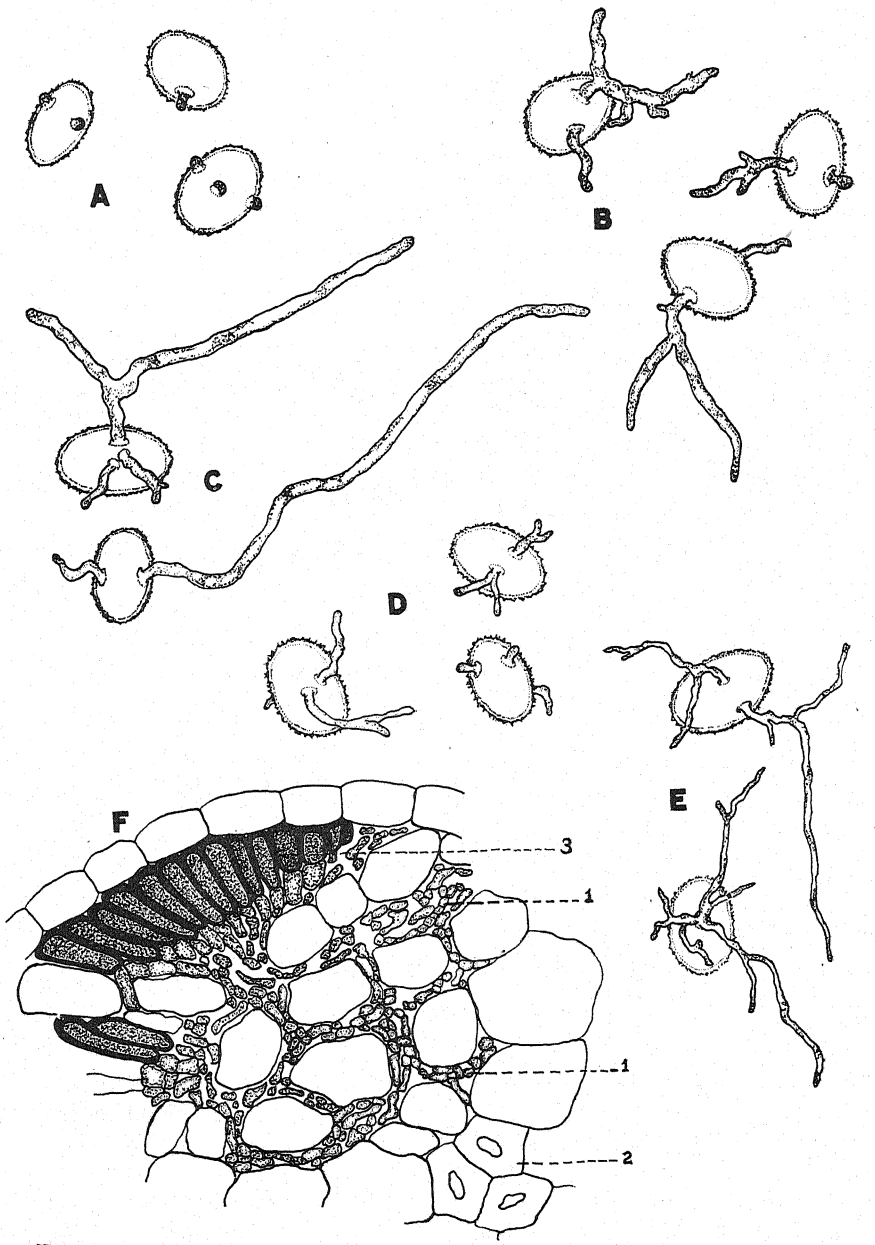

FIG. 1. A–E. Germinating urediniospores. A. After 1½ hours in distilled water at 18° C. B. After 4 hours in distilled water at 18° C. C. After 9 hours in distilled water at 18° C. D. After 24 hours at 2.5° C. E. After 48 hours at 25° C. F. Cross section of rusted stem of Winona, showing the intercellular hyphae (1) which have separated the cortical cells of the host and have penetrated as far as the bast fibers (2). A layer of young teliospores (3) has been formed just beneath the epidermis.

ditions, some of the germ tubes reach a length of 20 microns within 3 hours, and after 24 hours many are 150 microns long.

Contact with water is necessary for the germination of urediniospores. The writer never has observed their germination in a saturated atmosphere, while those floating on distilled water germinated readily. As in the case of aeciospores, the addition of host tissue to distilled water did not stimulate germination appreciably.

TABLE 1.—*The effect of temperature on the germination of aeciospores of Melampsora lini in distilled water*

Temperature in degrees C.	Percentage of germination	Character of germ tubes
0– 2	2.5	Very short and slender; weak
6– 7	46.5	Short, but fairly vigorous
11–12	81.5	Long and vigorous
17–18	90	Long and very vigorous
21–22	80	Long and vigorous
24–25.5	62	Short and very weak
28–29	0	

Oxygen is required for urediniospore germination. Spores floating on distilled water germinate readily, while those submerged in water, and therefore having a diminished supply of free oxygen, seldom germinate. From 60 to 80 per cent of spores germinated when abundant oxygen was available, while none germinated when all of the oxygen was removed by pyrogallol.

Temperature has a decided effect on the germination of urediniospores. The optimum is about 18° C., the same as for aeciospore germination. The highest percentage of spores germinate at the optimum, but fairly abundant germination and vigorous germ tube growth is obtained at temperatures ranging from 6° to 23° C. Beyond these limits the percentage of germination decreases, and germ tubes are short and weak (Fig. 1, D and E). Even at 23° C. the germ tubes are poorly developed. At 27.5° C. the spores did not germinate, the maximum temperature apparently being about 26° or 27° C. The minimum lies very close to 0° C., for a small percentage of spores germinate at from 0° to 1° C. In one instance, 9 per cent of the spores germinated during eight hours at from 0.5° to 2.5° C., but the germ tubes grow very slowly at such low temperatures (Fig. 1, D). Table 2 gives the percentages of urediniospore germination obtained at different temperatures in five representative trials.

Light seems to have little or no effect on the germination of the urediniospores of *M. lini*. Germination tests were made in light and darkness at

TABLE 2.—*The effect of temperature on germination of urediniospores of Melampsora lini in distilled water*

Series number	Temperature in degrees Centigrade								
	0.5–2.5	6.7	11–12	15–17	18–19	21–23	24.5–25.5	27.5	30
	Percentage of germination[a]								
1	–	37	38	45	–	54	–	0	0
2	–	62	65	78	87	50	16	0	–
3	–	41	55	47	–	56	9	–	0
4	9	60	75	–	87	73	6	0	–
5	tr	77	85	86	91	15	4	0	–
Average	5	55	64	64	88	50	9	0	0

[a] tr = less than 1%; – = no test made.

temperatures of 14°, 17°, and 23.5° C. In every case the development of the germ tubes and the germination percentages at a given temperature were approximately the same in light and darkness. Table 3 gives the averages obtained during three trials.

TABLE 3.—*The effect of light on the germination of urediniospores of Melampsora lini in distilled water at different temperatures*

Temperature in degrees C.	Light conditions	Percentage of germination
14	Artificial electric light	42
14	Darkness	45
17	Sunlight	62
17	Darkness	60
23.5	Sunlight	4
23.5	Darkness	5

Teliospores. Pethybridge, Lafferty, and Rhynehart (15) recently have described the germination of teliospores. Each spore produces a short, thick promycelium, usually divided into four cells by transverse walls. A single oval or pear-shaped basidiospore is borne on the sterigma of each cell.

Teliospores require a rest period before germination. The writer made several attempts to shorten the period of dormancy, but failed in all of them. Teliospores stored out-of-doors during the winter did not begin to germinate until early June. In 1924, teliospores began to germinate on June 12, and flax rust was first found in the field on June 20.

Thiel and Weiss (20) found that the rest period of teliospores of *Puccinia graminis tritici* (Pers.) Erikss. and Henn. could be shortened by treat-

ment with one per cent citric acid for fifteen minutes. The writer soaked teliospores of *Melampsora lini* in one per cent citric acid for periods varying from ten to thirty minutes, but the rest period was not shortened.

Noble (11) found presoaking necessary to the germination of spores of *Urocystis tritici* Koern. Teliospores of *Melampsora lini* were presoaked in distilled water for periods varying from a few hours to a week, at the end of which time flax tissue was added, but none of the spores germinated.

Alternate freezing and thawing did not shorten the period of dormancy, nor did lowering the surface tension by films of benzaldehyde, citral, oil of bergamot and oil of garlic have any effect. Exposure to ether vapor for periods varying from two seconds to two minutes also was ineffective. Solutions of sodium nitrate, calcium nitrate, and potassium nitrate were used in concentrations of N/1, N/10, N/25, and N/300, but none of the spores germinated. If the dormancy period can be shortened, the right methods for accomplishing it have not been found. As the methods which are effective in stimulating germination of spores of several other fungi were ineffective for teliospores of *M. lini,* it is possible that a period of real "after ripening" is necessary.

VITALITY OF SPORES

As the rust is spread largely by urediniospores, it is important to know how long they retain their viability.

Temperature and relative humidity are known to affect the vitality of spores. The effect of these two factors on the vitality of urediniospores was therefore investigated. Uredinial material from greenhouse cultures was kept at 7° C., 11° C., 17° C., and 22° C., in relative humidities of 0, 20, 40, 60, and 80 per cent. There was a plus and minus fluctuation of about one degree for the different temperatures. The relative humidity was controlled by using various concentrations of sulphuric acid. The material was suspended above the acid in sealed bottles kept at different temperatures. The results of the germination tests are given in table 4.

Seventy per cent of the spores germinated at the beginning of the experiment. As time passed, the germination percentages gradually decreased until none of the spores germinated. It will be seen from table 4 that the spores lost their vitality more rapidly at the higher temperatures than at the lower ones, and at the extreme relative humidities than at the intermediate ones. At the end of five weeks nearly all the spores kept at 22° C. had lost their viability, for less than one per cent of those kept at a relative humidity of 40 per cent germinated, and none of those kept at the other relative humidities germinated. After eleven weeks, all spores, except those kept at 7° C. and 60 per cent relative humidity, had lost their viability. After twelve weeks none of the spores germinated, although it is possible

that the urediniospores remain viable longer at still lower temperatures. However, this seems improbable; and it seems unlikely, from the results obtained, that the urediniospores retain their viability during the winter.

TABLE 4.—*The effect of temperature and humidity on the viability of urediniospores of Melampsora lini*

Temperature in degrees C.	Relative humidity in per cent	Time in weeks					
		1	3	5	7	11	12
		Percentage of germination in distilled water[a]					
7	0	tr	0	0	0	0	0
	20	38	11	7	2	0	0
	40	18	9	11	8	—	—
	60	52	6.5	16	12.5	tr	0
	80	40	7.5	10	2.5	0	0
11	0	2	1.2	tr	0	0	0
	20	42	10	3	1.5	0	0
	40	37.5	25	4	2	0	0
	60	38	tr	2	tr	0	0
	80	36	0	0	0	0	0
17	0	5	0	0	0	0	0
	20	25	9	5	tr	0	0
	40	24.5	3	tr	tr	0	0
	60	38	10	tr	0	0	0
	80	tr	0	0	0	0	0
22	0	—	—	—	—	—	—
	20	32	tr	0	0	0	—
	40	39	2	tr	0	0	—
	60	11.5	tr	0	0	0	—
	80	tr	0	0	0	0	—

[a] tr = less than 1%; — = no test made.

As is shown in table 4, the decrease in the percentage of spore germination is more gradual at low than at high temperatures. The largest number of spores seemed to remain viable for the longest period of time at 7° C. The most favorable relative humidity seemed to be 40 per cent in most cases, although at 7° C. a relative humidity of 60 per cent seemed most favorable.

INFECTION PHENOMENA

The uredinial germ tubes enter the host through the stomata. The manner of entrance is similar to that described by Stakman (18) for *Puc-*

cinia graminis tritici. The urediniospores of *M. lini* produce germ tubes which grow toward the stomata, following very closely the contour of the epidermis. The tube swells directly over the stomatal opening and the protoplasmic contents are concentrated in an appressorium (Fig. 2, B). A slender penetration tube then passes between the guard cells and forms a substomatal vesicle. Fig. 2, B, D, and E, illustrate the entrance of the pathogene in a susceptible host. From the substomatal vesicle numerous infection threads grow out in all directions, branching between the cells of the host and sending haustoria into them (Fig. 2, E). The invaded host cells appear to be uninjured for a considerable time after infection.

Miss Marryat (8) found that the uredinial germ tubes of *Puccinia glumarum* (Schum.) E. and H. entered highly resistant hosts as well as susceptible ones. Stakman (18), Newton (10), and Allen (1) found the same to be true of *Puccinia graminis tritici* (Pers.) E. and H. Flax rust is very similar, for the uredinial germ tubes enter the stomata of Argentine flax, a practically immune variety. There is no externally visible sign of entry, but it is evident from histological study that typical appressoria, penetration tubes, and substomatal vesicles are formed (Fig. 2, C). Infection threads grow out from the substomatal vesicle, and haustoria sometimes may be sent into the cells. The host cells seem extremely sensitive to the rust hyphae, and, within a week after inoculation, some of the host cells are killed by the fungus which is unable to establish itself and finally dies (Fig. 2, F). The action is usually very rapid, for only a few of the host cells are killed by the pathogene before it dies.

The time required for the entrance of the pathogene into the host was determined by varying the time during which inoculated plants were kept in the moist chamber. Fourteen pots of flax, of ten plants each, were inoculated and placed in the moist chamber at the same time. They were removed in pairs after intervals of 1½, 3, 6, 9, 14, 24, and 48 hours, respectively. Observations were made 10 days after inoculation. No uredinia appeared on plants incubated only 1½ hours. Even under optimum conditions, the urediniospores did not begin to germinate until after 1½ hours, so that no germ tubes were long enough to effect entrance when plants were kept in a moist chamber for only this length of time. The plants incubated for three hours became very slightly infected, 11 uredinia appearing on a total of 20 plants.

The germ tubes are often 20 microns long at the end of 3 hours, so that entrance may be effected very easily within that time if a spore is near a stoma on the leaf. Light infection developed on plants incubated in the moist chamber for 6 hours, and moderately heavy infection on those plants incubated 9 hours and 14 hours. Plants kept in the moist chamber for 24

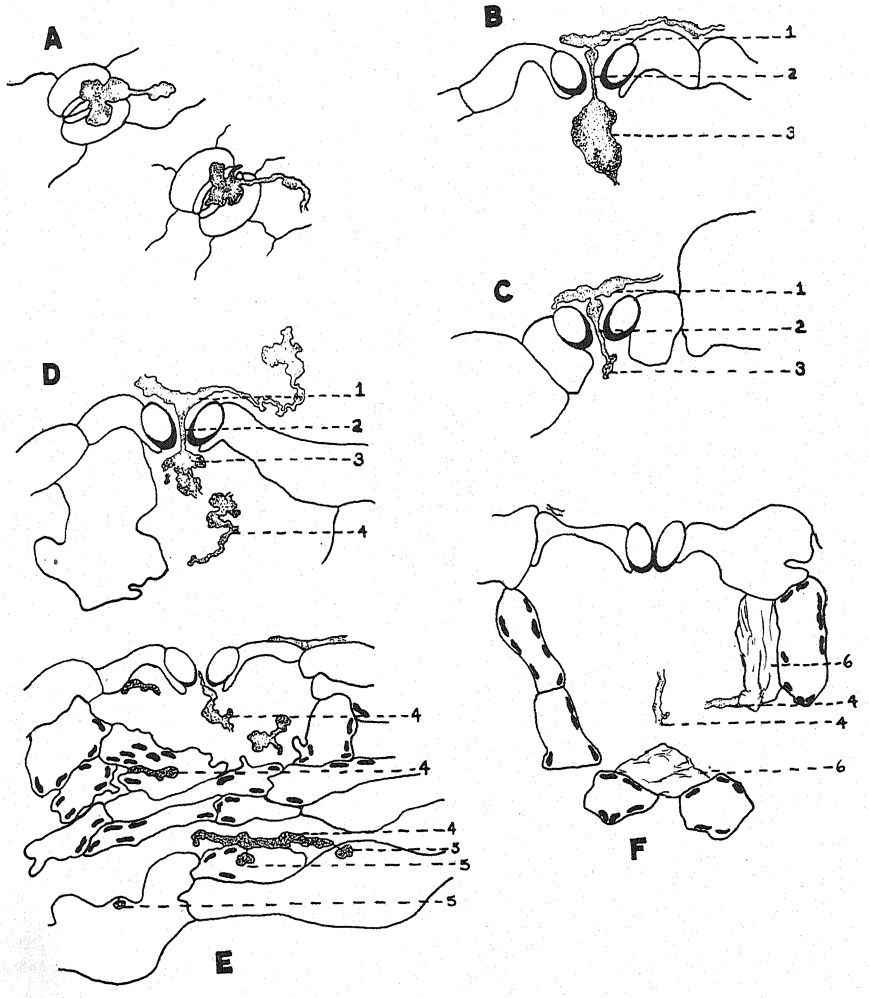

FIG. 2. A. Surface view of two appressoria formed by uredinial germ tubes over stomata. After forty-eight hours in the moist chamber. B. Section of a leaf of Winona flax, taken after forty-eight hours in the moist chamber, showing an appressorium (1) over the stoma, a slender penetration tube (2) passing through the stomatal opening and a sub-stomatal vesicle (3) formed beneath the stoma. C. Section of a leaf of Argentine flax, taken after forty-eight hours in a moist chamber, showing a typical appressorium (1), penetration tube (2) and small sub-stomatal vesicle (3). D. Section of Winona leaf, taken four days after inoculation, showing an appressorium (1), penetration tube (2), sub-stomatal vesicle (3) and the hyphae (4) developing from the sub-stomatal vesicle. E. Section of Winona leaf, taken five days after inoculation, showing the intercellular hyphae (4) which penetrate the host tissue and send haustoria (5) into the host cells. F. Section of Argentine leaf, taken seven days after inoculation, showing infection threads (4) which have destroyed certain host cells (6).

and 48 hours became heavily infected. The fact that germ tubes may enter in as short a time as 3 hours indicates that a small amount of moisture for a relatively short time may be sufficient for the process in the field.

FACTORS AFFECTING LENGTH OF INCUBATION PERIOD

In the greenhouse, at temperatures between 16° and 23° C., uredinia appear 9 or 10 days after inoculation. The first evidence of infection is the appearance of flecks, minute circular areas lighter in color than that of the normal, green leaf. The light areas soon take on an orange color, owing to the formation of urediniospores beneath the epidermis of the host. Within 24 hours the epidermis is ruptured by the fully formed uredinium.

The length of the incubation period is greatly influenced by external conditions. The effect of environment on the incubation period of the uredinial stage was studied most extensively. It was found that environmental conditions immediately following inoculation affect the germination of spores and the entrance of the pathogene, and, after the fungus enters its host, environmental conditions are important in determining the rapidity of development and the severity of the infection. Factors influencing spore germination may be relatively unimportant in uredinial formation and *vice versa.*

Moisture. As shown above, no rust develops on plants supplied with abundant moisture for only 1½ hours. Abundant moisture for 3 hours, immediately after inoculation, is sufficient to enable a few spores to germinate and send germ tubes into the host, but severe infection is obtained only when the time is extended to 24 hours.

Light. Light has virtually no effect on the germination of the spores of *M. lini,* and evidently has no influence on the entrance of the germ tubes. One series of plants was inoculated with urediniospores and placed in a moist chamber exposed to sunlight during the day and to artificial light during the night. A second series, inoculated at the same time, was placed in a moist chamber from which all light was excluded by a cover of six layers of heavy wrapping paper. The temperature was favorable to spore germination, varying from 17° C. to 23° C. After 48 hours, the plants of both series were removed from the moist chambers and placed on the greenhouse bench. On the 11th day uredinia appeared in both series, and the degree of infection was approximately the same, indicating that the germ tubes are able to enter the host equally well in darkness and in light.

Light appears to have little effect on germination of the spores and subsequent entrance of the germ tube into the host, but it is essential to pustule formation after the pathogene has established itself within the host. On plants exposed to continuous electric light with an intensity of 400 to 600

foot candles at the leaf surface (a low intensity when compared with that of sunlight), uredinia appeared 6½ days after inoculation, while check plants, under normal conditions of alternate sunlight and darkness, did not fleck until the 9th day after inoculation. While increased light shortens the incubation period, decreased light tends to lengthen it, and retards the formation of uredinia. On plants shaded with black cambric and placed in a dark corner of the greenhouse, uredinia did not develop until the 14th day after inoculation, 5 days later than on the check plants exposed to full sunlight, and almost 8 days later than on plants exposed to continuous electric light.

Temperature. The influence of temperature during the moist chamber period was determined by placing inoculated plants in moist chambers kept at different temperatures. All the plants were inoculated under uniform conditions and one-third of the lot placed in a moist chamber at 4–10° C., one-third at 16–20° C., and the remaining third at 27–30° C. After 48 hours, all were removed to the greenhouse bench and kept at a temperature of 16–22° C. under optimum light conditions. The first uredinia appeared 9½ days after inoculation, on the plants which had been kept in the moist chamber at 4–10° C. Ten days after inoculation, uredinia appeared on the plants incubated in the moist chamber at 16–20° C. None of the plants in the third series became infected, the temperature being too high for the germination of spores. The results of the experiment agree with the results of spore germination tests, for the urediniospores germinate readily at temperatures between 6° and 22° C., while the maximum temperature for germination is about 26° or 27° C.

Uredinia may form within a fairly wide range of temperatures, but the optimum coincides with the optimum temperature for spore germination and that of the growth of the flax plant. Nine pots of flax were inoculated and placed in a moist chamber at from 16° to 20° C. After 48 hours, they were subjected to different conditions. Three pots were exposed to temperatures of from 7° C. to 14° C., three were placed at 16° to 22° C., and three at 25° to 30° C. The results are summarized in table 5. The lower and higher temperatures retard the formation of uredinia. At 16° to 22° C., uredinia first appeared on the 10th day after inoculation. On the 11th day, the flecks and uredinia were numerous, and from 65 per cent to 100 per cent of the plants were infected. On the 14th day the rust infection was severe, the uredinia being large and very numerous. At the lower temperatures, 7–14° C., the first uredinium appeared 14 days after inoculation, and at the higher temperatures, 25–30° C., uredinia appeared on the 15th day. Infection was always light at the lower and higher temperatures; the uredinia were always small and relatively few in number.

TABLE 5.—*The effect of temperature on the development of uredinia of Melampsora lini on Winona flax, C. I. 179, inoculated with urediniospores and kept in a moist chamber for 48 hours at 16°–20° C.*

Temperature during period of incubation		Series no.	Number of days after inoculation and degree of infection							
Moist chamber	After removal		10	11	12	13	14	15	16	17
	7–14° C.	1								1 uredinium
		2						Cotyledons of 1 plant heavily infected	Numerous uredinia and flecks	
		3							2 small uredinia	Very light
16–20° C.	16–20° C.	1	2 uredinia, no flecks	11 uredinia, numerous flecks, 66% plants infected	Light		Moderate	Moderate	Heavy	Heavy
		2	2 uredinia, numerous flecks	Uredinia and flecks numerous. 100% plants infected	Moderate	Moderate	Heavy	Heavy	Heavy	Heavy
		3	1 uredinium, few flecks	Uredinia and flecks numerous. 80% plants infected	Moderate	Moderate	Heavy	Heavy	Heavy	Heavy
	26–30° C.	1								1 uredinium
		2					1 uredinium	1 uredinium, flecks	Light	Light
		3						1 uredinium		Very light

THE EFFECTS OF SOIL NUTRIENTS ON RUST INFECTION

Soil nutrients influence the growth and vigor of the host plant and thus indirectly influence the development of the rust. Obligate parasites, such as *M. lini,* usually thrive best on healthy plants. In order to ascertain the effect of nutrients, Winona flax, C. I. 179, was planted in sterilized soil and supplied with nutrient solutions of calcium acid phosphate, magnesium acid phosphate, potassium nitrate, sodium nitrate, and potassium sulphate made up in the following proportions:

2.944 gm. $CaHPO_4$	1000 c.c. distilled H_2O
2.980 gm. $MgHPO_4$	do
1.728 gm. KNO_3	do
1.452 gm. $NaNO_3$	do
2.980 gm. K_2SO_4	do

Check plants were supplied with distilled water.

Inoculations were made six weeks after planting. At that time, the plants in the acid phosphate series were larger and more vigorous than the others. The leaves were large and bright green, and nearly every plant had developed several branches. Plants in the nitrate series grew moderately well, developing strong, erect stems which seldom branched. The leaves were smaller and darker in color than those in the phosphate series. Plants supplied with sulphate grew very slowly. The leaves were very small, and the plants were about half as high as those in the phosphate or nitrate series. Even the check plants appeared more vigorous than those supplied with sulphate.

The plants were inoculated with urediniospores of *M. lini,* kept in a moist chamber for 48 hours, and then removed to the greenhouse bench. Observations were made on the 10th day after inoculation and are given below.

Phosphate series: Uredinia numerous, large, and fully formed, with the ruptured epidermis of the host evident. Slightly greater infection on plants in the $MgHPO_4$ series than those in the $CaHPO_4$ series.

Nitrate series: Uredinia numerous but not large; some uredinia fully formed, while others were just rupturing the epidermis of the host. Somewhat greater infection on the $NaNO_3$ series than the KNO_3 series.

Sulphate series: Uredinia few and very small; just breaking the epidermis of the host.

Check plants: Uredinia few and small.

Uredinia seem to appear sooner, and in greater abundance, on the most vigorous plants, a result which is to be expected. Pethybridge, Lafferty and Rhynehart (15) state that the intensity of a flax rust attack appears to increase with the luxuriance of host growth. Observations were again

made 17 days after inoculation, and those plants furnished with phosphates and with nitrates were very severely infected. There was very light infection, and practically no injury, on plants in the sulphate series and in the distilled water series. The plants in the phosphate series always were most severely infected.

HISTOLOGICAL RELATIONS BETWEEN HOST AND PATHOGENE

Rust attacks all the above-ground parts of the flax plant: stems, cotyledons, leaves, pedicels, and sepals. Early in the season, when the uredinial stage is prevalent, the rusted leaves are most conspicuous, but later, after the development of teliospores, the rust appears to be most abundant on the stems. It is the stems of fiber flax which are damaged, a moderate amount of rust causing a great deal of injury. The rust on the leaves is just as injurious to seed flax as that on the stems; the leaves often are killed and the entire plant may be stunted, so that it yields poorly. Heavy infection causes the stems to become so brittle as to break at the point of attack.

The hyphae are intercellular, pushing their way between the host cells. Very often the mycelium is so abundant that the cells of the host are wedged apart and separated from each other (Fig. 1, F).

In all of the sections of infected flax stems examined, the mycelium seemed to be restricted to the cortical region. None of the hyphae were ever seen penetrating the woody portion of the stem. In most of the sections they had penetrated as far as the bast fibers, which are grouped in strands in the cortex. The fibers themselves are often surrounded by the mycelium. Tobler (21) has described the effect of the fungus on the bast fibers and states that often they are more severely attacked than any of the other tissues. He found that in some cases the pathogene seems to modify the wall substance so that the thick walls of the fibers become much thinner. When the rust is severe, the fibers collapse and shrivel as though the cells of the adjoining cortex had exerted a pressure upon them. Tobler states that in some cases the fibers appear to be lignified when attacked by the rust. Also the pathogene seems to have a preservative effect, protecting the tissues from decay, so that the fiber cannot be separated in the retting process which fiber flax must undergo.

Fiber flax undoubtedly is severely injured by the rust. Seed flax suffers little damage until the mycelium becomes abundant enough to kill the tissues or retard the growth of the plant. The attack on the stems causes them to become brittle and break easily, a condition which may result in a poor stand and decreased yield. The destruction of the fibers in seed flax is not of such direct importance as in fiber flax.

PHYSIOLOGIC SPECIALIZATION

Persoon (13, p. 216), in 1801, described a rust of flax collected on *Linum usitatissimum* L. and *L. catharticum* L., in which the uredinial stage was noted and designated as *Uredo miniata* var. *lini* Pers. Since that time, several authors have described flax rust, nearly every one placing the causal organism in a different genus. It has been described as *Uredo, Podosporium, Lecythea, Xyloma,* and *Podocystis,* but it was not until 1847 that Léveillé (7) placed the fungus in the genus *Melampsora.*

According to Saccardo (17), *M. lini* occurs on the following species of *Linum: L. catharticum* L., *L. usitatissimum* L., *L. narbonense* L., *L. nodiflorum* L., *L. marginale* A. Cunn., *L. alpinum* Jacq., *L. perenne* L., *L. strictum* L., and *L. austriacum* L.

Sydow (19) reports the occurrence of rust on these additional species of *Linum: L. anatolicum L. breweri, L. capitatum, L. congestum, L. corymbulosum, L. drymarioides, L. gallicum, L. hirsutum, L. lewisii, L. pubescens, L. possariodes, L. rigidum, L. sulcatum,* and *L. viscosum.*[2]

In the United States, Arthur (3) reports rust on *L. breweri* A. Gray, *L. drymarioides* Curran, *L. rigidum* Pursh., and *L. sulcatum* Redd.

Körnicke probably was the first to suggest the possibility of specialization within *Melampsora lini.* According to Bucheim (4), Körnicke pointed out, as early as 1865, that in Prussia the rust on *Linum usitatissimum* was a special variety which he named *M. lini* var. *liniperda.* Körnicke also pointed out that *Linum usitatissimum* often was free from rust under conditions where *Linum catharticum* rusted commonly.

In 1910, Palm (12) found that *M. lini* on *Linum usitatissimum* had much larger teliospores than those described for *M. lini* on *L. catharticum.* His attempts to infect *L. catharticum* with urediniospores from *L. usitatissimum* were unsuccessful. His results led him to regard the form on common flax as a distinct species, and he named it *Melampsora liniperda* (Körnicke) Palm.

In 1915, Eriksson (5) concluded that there were two forms of *M. lini,* one on common flax, *Linum usitatissimum,* and one on *L. catharticum.*

In 1915, Bucheim (4) made cross inoculations with urediniospores of *M. lini* on four different species of *Linum; viz.*: *L. catharticum, L. alpinum, L. strictum,* and *L. tenuifolium.* The results indicated that the rust on each of the four hosts was highly specialized, infecting its own particular host but being unable to infect other species of *Linum.*

Pethybridge, Lafferty, and Rhynehart (16), in 1922, failed to infect common flax with rust obtained from purging flax, *L. catharticum.*

Wild flax, *L. lewisii,* is common in the Dakotas and Montana, and is frequently rusted. If the forms of *M. lini* on the common flax, *L. usitatis-*

[2] The authors of these species are not given by Sydow.

simum, and the wild flax, *L. lewisii,* were the same, the wild flax might be important in spreading rust to the cultivated flax. Arthur (2) states that teliospores of *M. lini* from common flax infected *L. lewisii,* an indication that the rust is not specialized to a particular species in this country.

The writer attempted to ascertain to what extent physiological specialization occurs in *M. lini.* Winona flax, susceptible to the rust of common flax, was inoculated with urediniospores taken from the wild flax, *L. lewisii.*[3] No infection resulted on two lots of plants inoculated in the first series, but four small uredinia appeared on plants in the other two lots. At first it was thought that a successful transfer had been accomplished, but in view of the later experiments it is probable that accidental infection with urediniospores of rust from common flax had taken place, inasmuch as the plants were not as completely isolated as in later experiments. All subsequent attempts to cause infection were unsuccessful. It appears, therefore, that the rust on *L. lewisii* is specialized for its host and does not attack Winona, a susceptible variety of common flax.

In addition to Winona, the resistant Argentine was inoculated with urediniospores from *L. lewisii.* No infection resulted in any case. While Bucheim showed that the rust of common flax does not attack *L. catharticum, L. alpinum, L. strictum,* nor *L. tenuifolium,* in Europe, no reports other than Arthur's (2) have been made regarding its ability to attack the species common in the flax-growing areas of the United States. Therefore attempts were made to transfer the rust from common flax to *L. lewisii* and *L. rigidum,*[4] the two wild species which are probably most common in the western areas. Seed of *L. sulcatum,* a species common in Minnesota and Wisconsin, was collected, but it germinated so poorly that plants for only two inoculations were obtained. Infection did not occur in either case. The *L. lewisii* used in the experiment came from some plants collected the previous fall. New shoots put out by the perennial crown were grown in the greenhouse for three months. No rust developed on them, thus indicating probable freedom from previous infection. When *L. lewisii* was inoculated with urediniospores from common flax, the results were always negative. Only three trials could be made with *L. rigidum.* At the time of inoculation the plants were very small, there being only three or four leaves on each one. No infection was secured in the first trial, but in the second and third trials *L. rigidum* was severely infected with rust from common flax. The incubation period was somewhat longer than it usually

[3] Specimens of rusted *L. lewisii* sent by Professor E. B. Payson and by Mr. R. U. Cotter, of the University of Wyoming.

[4] Plants of *L. lewisii* were obtained from Mrs. F. M. Heath, through Professor Baird, University of North Dakota. Seed of *L. rigidum* was furnished by Professor E. J. Petry, State Agricultural College, Brookings, South Dakota.

is on common flax, as the uredinia did not develop until the 15th day after inoculation. After 20 days, the plants were severely rusted.

Apparently, *L. lewisii* need not be regarded as a source of infection of cultivated flax, for the rusts on the two species seem to be highly specialized; on the other hand, *L. rigidum* may prove dangerous, for the rust on common flax attacks this wild species. It was impossible to obtain naturally rusted *L. rigidum,* but it is quite probable that the rust can be transferred from this wild species to cultivated flax.

According to our present knowledge, the variety Argentine is virtually immune from rust, but it is possible that there is some form which can attack it. Rust on common flax, therefore, was collected from several different localities and cultured in the greenhouse. The following strains were maintained successfully: (1) from Purgatory Plats, Hennepin County, Minn.; (2) from University Farm, St. Paul, Minn.; (3) from Fargo, N. D.; (4) from Mandan, N. D.; (5) from Indian Head, Saskatchewan, Canada; (6) from Saskatoon, Sask., Canada. Argentine and Winona were inoculated with urediniospores from each of the rust collections. Host plants of various ages were used in order to determine whether the age of the host had any effect on its resistance. Plants of various ages, up to maturity, were inoculated. Winona became infected with every culture, the degree of infection ranging from moderate to heavy, but not varying much according to the age of the host. In most cases where Argentine was inoculated, the results were entirely negative, no signs of infection appearing. But in one experiment, two small flecks appeared on one plant out of six which had been inoculated with the culture from Mandan. The flecks developed into aborted uredinia which formed spores but never broke through the epidermis of the host. The uredinia remained in such a condition for two weeks, when the epidermis was ruptured with a needle and the spores transferred to other leaves of the same plant. Following the second inoculation, two very small uredinia appeared, but the leaves shriveled before the uredinia were fully formed, and the rust was lost. All other trials with the Mandan strain gave negative results. In one other experiment, infection resulted when Argentine was inoculated with urediniospores of the Fargo strain. A small uredinium appeared on one plant in twelve. In this case the uredinium was fully formed and the epidermis of the host was ruptured. A few days later several uredinia appeared on the same plant; and, two weeks after the first uredinium was noticed, the plant was heavily rusted. There were no signs of infection on any of the other plants even when inoculated with spores from the rusted Argentine plant. As only a single plant was rusted, it seemed probable that it may not have been Argentine. This explanation seems most plausible, because the experiment was

repeated many times, always with negative results. None of the other strains infected Argentine. This does not prove that there is no further specialization within *M. lini* on varieties of common flax, but it does indicate that, if there are physiologic forms, probably none of those occurring in this region are able to attack Argentine. The results are important when one considers the fact that Argentine is being used extensively as a resistant parent in flax breeding studies.

CONCLUSIONS

The facts obtained in these investigations indicate that epidemics of flax rust may occur each year under normal conditions. Cool, moist weather, the most favorable condition for the growth of flax, also is conducive to the development of flax rust. Spores germinate within a wide range of temperature. The spread of the disease is checked by hot, dry weather, as the maximum temperature for spore germination is 26–27° C. Uredinia are formed over a wide range of temperature, so that the supply of inoculum may increase rapidly, even at fairly low or high temperatures. Low temperature checks the development of the disease only slightly, since it merely delays the production of inoculum and does not inhibit spore germination. High temperature delays the formation of uredinia and inhibits spore germination so that the inoculum produced is not effective until a return of favorable conditions.

Spores must be in contact with water in order to germinate, but the fact that the pathogene can enter the host when abundant moisure is supplied for only three hours indicates that either light showers or dews may furnish sufficient moisture for infection. If plants are wet for only a short time, therefore, they may become infected.

Light seems to have little effect on spore germination and entrance of the germ tubes into the host. Infection can occur at any time of night or day. However, light is required for the formation of uredinia. Fair days favor the formation of uredinia, and a succession of cloudy days lengthens the incubation period somewhat; but apparently there is always enough light to enable the rust to develop well in nature.

The fact that *M. lini* is not restricted to common flax suggests the possibility that wild flaxes may be dangerous sources of inoculum in flax-growing areas. So far as determined, *L. lewisii* need not be feared, for the rust occurring on it probably will not attack the cultivated species. On the other hand, the wild species, *L. rigidum*, is attacked by the rust from common flax and may prove to be a source of infection for the cultivated crop.

The use of resistant varieties affords the only practical means of controlling flax rust. So far, Argentine has proved to be practically immune

from rust, apparently possessing a true physiologic resistance. Uredinial germ tubes of the fungus enter the resistant Argentine as well as the susceptible Winona flax. Argentine is hypersensitive to the pathogene, which kills some of the host cells quickly and is then unable to develop further. The evidence obtained by the writer indicates that in the principal flax-growing states there are no physiologic forms which can attack Argentine.

SUMMARY

1. A study was made of the factors affecting infection and development of *Melampsora lini* (Pers.) Lev.

2. The cardinal temperatures for the germination of aeciospores and urediniospores are 0.5° C., 18° C., and 26–27° C..

3. Aeciospores and urediniospores germinate readily in distilled water. The presence of host tissue does not stimulate germination appreciably.

4. Aeciospores begin to germinate at the end of 45 minutes at optimum temperature, each spore sending out from one to six germ tubes.

5. Urediniospores begin to germinate after 1½ hours, each spore sending out one or more branched, reddish-orange germ tubes, one of which develops more rapidly than the rest.

6. Urediniospores germinate equally well in light and in darkness.

7. Under favorable conditions, urediniospores retain their viability almost three months.

8. Urediniospores lose their viability more rapidly at high temperatures than at low ones.

9. Urediniospores retain their viability longer at relative humidities of 40 per cent and 60 per cent than at relative humidities of 20 per cent and 80 per cent.

10. Teliospores require a rest period before germination. Attempts to shorten the rest period failed.

11. Uredinial germ tubes enter the host through the stomata.

12. The uredinial germ tubes enter resistant hosts as well as susceptible ones, but the pathogene kills some of the cells of resistant hosts and then cannot develop further.

13. Uredinial infection occurs if inoculated plants are kept in moist chambers for 3 hours or more.

14. Light is necessary for the formation of uredinia. Increased light hastens formation of uredinia, while decreased light lengthens the incubation period.

15. Uredinia appear at temperatures ranging from 7 to 30° C. Severe infection occurs at 16–22° C. Slight infection occurs at 7–14° C., and at 26–30° C.

16. The intensity of infection is directly proportional to the luxuriance of growth of the host. Plants supplied with phosphates grew more luxuriantly and were more severely rusted than plants supplied with nitrates or sulphates.

17. The pathogene penetrates the cortical tissues of the flax and often attacks the fibers. The hyphae do not extend into the xylem.

18. Urediniospores of *Melampsora lini* from common flax do not infect the wild flax, *Linum lewisii*; and urediniospores from *L. lewisii* do not infect the common flax.

19. Urediniospores of *M. lini* from common flax infect *L. rigidum* heavily.

20. The variety Argentine was immune from all the strains of *M. lini* used in these studies.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY FARM, ST. PAUL.

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RELATION OF INTERNAL COB DISCOLORATION TO YIELD IN CORN

R. A. JEHLE, F. W. OLDENBURG, AND C. E. TEMPLE

The diseases of corn referred to by Holbert and Hoffer (1, 2) as the root, stalk, and ear rot diseases are very prevalent in Maryland. The estimated annual loss to the corn growers of the state from these diseases is about 10 per cent of the crop, which amounts to approximately 2,500,000 bushels. These diseases are prevalent in all parts of Maryland, but they cause the greatest losses in the poorer sandy and clayey soils.

Plans were made in 1921 to conduct demonstrations in the control of the root, stalk, and ear rot diseases, following the recommendations of Holbert and Hoffer (1, 2). It was found that only a few of the corn growers were willing to use the germinator to test all of their seed ears; on the other hand, many of them were already following most of the other recommendations of Holbert and Hoffer, with the exception of discarding ears with discolored butts.

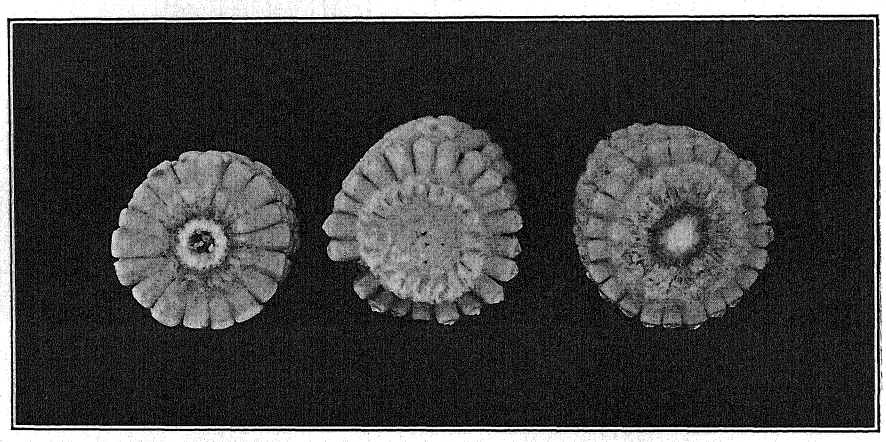

FIG. 1. An ear of corn practically free from internal cob-discoloration (center), and two ears showing internal cob discoloration. The center of the ear at the left is also hollow. The discolorations may be red, pink, gray, or brown; they may involve only the vascular tissue, or may extend to the surrounding tissues.

When the first seed corn selections were made in this project, some of the ears which showed no butt discoloration were broken, and discoloration

was found in other parts of the cob. It was soon found that many cobs were discolored in the interior without showing any discoloration at the butt. The butts and tips were then chopped from a number of ears; some were found to be discolored internally at the butts and not at the tips, some at the tips and not at the butts, and others were discolored all the way through, from butt to tip.

Plans were made for conducting a series of tests to determine whether the red, pink, gray, brown, and other discolorations found in the vascular and surrounding tissues of the interior of the cob were in any way related to yield and to root, ear, and stalk rot diseases (Fig. 1); and, also, whether

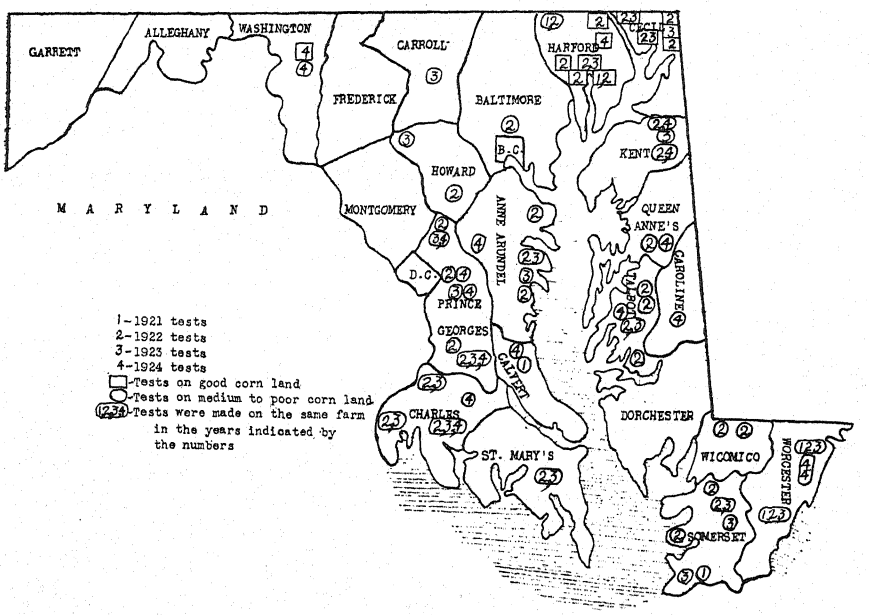

FIG. 2.—Location of corn root rot tests.

freedom from these internal cob-discolorations is an inherited character. These tests have been made in various parts of the State (Fig. 2) during the past four years.[1] They represent many different soil types and a wide range of climatic conditions. In some tests, pure-bred varieties, such as Reid's Yellow Dent, Improved Lehming, Collier's Excelsior, Johnson County White, and Boone County White were used; in other tests, mixed white and yellow dent varieties were used. In only one test, conducted on the Maryland Experiment Station grounds, were yield records obtained

[1] The writers were assisted in conducting these tests by other members of the Departments of Pathology and Agronomy and by the county agents.

with sweet corn. In addition to 90 tests in which accurate yield records were obtained, many other tests were made in which the yield records were estimated and field observations were recorded. In almost all of these additional tests, the results reported by the county agents and the growers were similar to those of the tests in which accurate records were obtained.

TABLE 1.—*Relation of internal cob-discoloration to yield in corn*

Year	No. of tests	No. of Counties	Av. yield of corn from cob without discoloration	Av. yield of corn from discolored cob	Average increase per cent	Average increase per cent	No negative results
1921 total	6	4	51.49	39.57	11.92	30.10	0
1922 total	39	15	67.19	58.37	8.82	15.11	4
1923 total	23	12	62.39	55.23	7.16	12.97	1
1924 total	25	11	55.18	46.45	8.73	18.79	2
1921 V.G.*	1	1	63.3	60.4	2.9	4.8	0
1921 M.P.**	5	4	49.03	35.41	13.62	38.46	0
1922 V.G.	9	3	88.91	82.66	5.63	6.81	2
1922 M.P.	30	15	60.46	51.08	9.38	18.36	2
1923 V.G.	3	2	81.42	78.25	2.57	3.28	0
1923 M.P.	20	11	59.49	51.78	7.71	14.90	1
1924 V.G.	2	2	63.45	61.52	1.93	3.13	1
1924 M.P.	23	10	54.46	45.14	9.32	20.64	1

(V.G.*) Very good corn land.
(M.P.**) Medium to poor land.

PROCEDURE

In each test, seed corn which had been selected by the grower was carefully culled. Off-type ears were discarded, as well as ears with moldy cobs or kernels; shredded shanks; blistered, checked, dull, soft, or starchy kernels; and discolored germs.

Instead of shelling the butts and tips, which is the usual practice, they were cut off. After the removal of the butts and tips, further examination of the grains was made for undesirable qualities. The selected ears were then divided into three or more lots, according to the intensity of internal cob-discoloration. The ears showing little or none were put into lot 1; those showing slight discoloration were put into lot 2; and those showing intense discoloration were put into lot 3. The amount of internal cob-

discoloration was found to vary greatly in different strains of corn, but the average amount which could be placed in lot 1 was approximately 10 per cent. A few strains were found in which 80 per cent of the ears could be placed in lot 1; whereas other strains were found in which practically all of the ears had medium to intense internal cob-discoloration.

In every test, a plot of lot 1 and another of lot 3 were planted. In some tests a plot of lot 2 also was included. A special effort was made to plant the corn where soil, drainage, and other conditions were uniform. Yield records were computed by weighing the corn from equal areas (about one-fourth acre) in each plot.

RELATION OF INTERNAL COB-DISCOLORATION TO YIELD

Yield records were obtained in 89 tests with field corn, and in one test with sweet corn. In 86 of these tests the corn planted from ears with practically no internal cob-discoloration gave a larger yield than the corn planted from ears with intense internal cob-discoloration. The results given in tables 1 and 2 show a decided correlation between internal cob-discoloration and yield, and this is more pronounced on sandy and clayey soils than on the best corn land.

TABLE 2.—*The relation of internal cob-discoloration to yield in sweet corn*

Internal cob-discoloration	Yield per acre in pounds	Total weight of dry ears in pounds	Increase over ears with most internal cob-discoloration	
			Pounds per acre	Per cent
Practically none	95.4	1431	176	14
Medium	90.8	1362	107	8.5
Intense	63.7	1255		

In some of the tests, three or four plots were planted, according to the intensity of the internal cob-discoloration. In almost every case there was an apparent correlation between the intensity of internal cob-discoloration and the yield. Some of the more striking instances are indicated in table 3.

INTERNAL COB-DISCOLORATION REDUCED BY SELECTION

In a large number of the tests, the selection for freedom from internal cob-discoloration was continued with the same corn grower for a period of from three to four years. In every instance the percentage of ears with internal cob-discoloration, as well as intensity of internal cob-discoloration,

was reduced by selection. Each year the seed ears were selected from the plots grown from ears with the least internal cob-discoloration. These seed ears were then sorted according to the intensity of cob-discoloration. Some of the most striking results are summarized in table 4.

INTERNAL COB-DISCOLORATION

The cause and nature of internal cob-discoloration have not been determined by the writers, but a number of investigators have made observations and studies which have a bearing on the subject. Holbert and Hoffer (2) state that "most ears have shank attachments pink, slightly pink or brown colored, or cracked or shredded, have grown on stalks more or less diseased," and that "such ears usually are diseased." In reference to the corn root rot diseases, Manns and Adams (3) state that "other symptoms

TABLE 3.—*The relation of intensity of internal cob-discoloration to yield in field corn*

Year	County	Plot and cob-discoloration	Bushels per acre	
			Yield	Yield from No. 1
1921	Harford	1	68.0	
		2 +[a]	50.8	17.2
		3 ++	42.6	25.4
1922	Anne Arundel	1	60.1	
		2 +	50.0	10.1
		3 ++	41.66	18.44
1922	Talbot	1	73.4	
		2 +	61.61	11.79
		3 ++	53.91	19.49
		4 +++	52.56	20.84
1923	Kent	1	54.48	
		2 +	50.30	4.18
		3 ++	34.74	19.74
1924	Anne Arundel	1	38.2	
		2 +	23.0	15.2
		3 ++	23.0	15.2

[a] + = relative amount of internal cob-discoloration.

which may indicate infection are discolorations of the cob at the butt or in the center. Such discolorations may be pink, brown, black, or yellowish." Kiesselbach (4) made germination tests from ears with yellow, pink, brown, and normal shanks in order to determine the percentage of kernels which were diseased and rotting after testing. He concluded that brown shank

discoloration was somewhat indicative of the corn root rot disease, and that pinkish and yellowish shank discolorations were not. Hoffer and Carr (5) found that the brown to brownish-purple discolorations which occur in the vascular plate tissue in the nodes of the stalk and shank and the scutellum of the kernels could be produced artificially by injecting solutions of certain iron and aluminum salts into the plants, and that "these artificially induced

TABLE 4.—*The effect of seed selection on the amount of internal cob-discoloration in corn*

Year	County	Per cent of seed ears with little or no internal cob-discoloration	Per cent of seed ears with internal cob-discoloration
1922	Cecil	75	25
1923		80	20
1924		67	33
1922	Charles	98	2
1923		90	10
1924		45.2	54.8
1922	Charles	95	5
1923		93.7	6.3
1924		56.8	44.2
1921	Carroll	85	15
1922		80	20
1923		70	30
1924		50	50
1922	St. Mary's	93	7
1923		70	30
1924		58	42
1921	Worcester	95	5
1922		80	20
1923		75	25
1924		3	97

changes in the plant parts closely resemble the phenomena which develop in the plants growing in fields under conditions favorable to the root rots." They also concluded that "corn plants showed marked differences in the quantities of aluminum and iron salts which are absorbed by them" and "these differences develop when the salts of iron and aluminum are available in subtoxic concentrations in the soil." These differences "are believed to be due to specific selective capacities of different plants to absorb

available aluminum and iron salts from the soil." They state that "resistance to the root rots in corn plants can be defined, first, by the respective abilities (inherited) of the plant to absorb materials present in the soil solution, and, second, by the relative concentrations of deleterious ions presented to the roots in soil solution."

Holbert, Burlison, Koehler, Woodworth, and Duncan (6) state that "there is much variation in color and in intensity of color of cob interiors either when viewed in cross-section after the cobs have been broken or when viewed in longitudinal section after they have been sawed longitudinally. Interior cob-coloring variations prevail in most open-pollinated strains of corn, even in those that have been selected rather closely for several years. It seems probable in many cases that red, pink, and brown colorings in the shank are due simply to lodgment in the shank attachment of the same pigment found in the vascular cylinder of the cob, and do not indicate infection. Hence, in observing the nature of the shank attachment for the selection of seed, it is very important to distinguish between normal colorations and discolorations resulting from decayed tissues. In describing a plate[2] in which internal cob-discoloration is illustrated, they state that "in the coloring of the interior of the cob, considerable natural variation occurs. In some cases the coloration extends to the point of attachment between the cob and shank, and thus gives the shank attachment a pink or brown appearance. In such cases the pink or brown shanks are normal." Thus they seem to be of the opinion that the internal cob-discolorations are normal when they do not result from infection and when they extend to the point of attachment between the cob and shank. Since the vascular system in the cob is a continuation of the vascular system in the shank, the internal discolorations which extend to the point of attachment between the cob and shank would seem to be caused by the same iron and aluminum compounds as those described by Hoffer and Carr (5). According to Holbert et al (6), the internal discolorations which do not extend to the point of attachment between the cob and shank result from infection by various organisms. If these conclusions are correct, internal discolorations resulting from both causes are being discarded by the Maryland method of chopping off the butts and tips and examining the interior of the cob at both ends.

In some of our tests, the symptoms attributed by several investigators to the root, ear, and stalk rot diseases, such as small and less vigorous plants, fallen and broken stalks, uneven stand, premature death, broken shanks, etc., were less pronounced in the plot grown from ears with least internal cob-discoloration than in the plots grown from ears with most internal cob-

[2] Plate V, page 378.

discoloration. This is very well illustrated by one test made in Harford County, where 248 down stalks were found in 400 hills in the plot grown from seed produced on ears with the most internal cob-discoloration; whereas only 64 down stalks in 400 hills were found in the plot grown from seed produced on ears with the least internal cob-discoloration. Similar conditions were observed in many other tests.

On a farm in Cecil County, a strain of yellow dent corn was found in which approximately 75 per cent of the ears were practically free from internal cob-discoloration. It has other desirable qualities, among which are a medium-sized cob and grain of a deep yellow color. This strain of corn has been planted in various parts of the state. In most places it has produced a uniform stand relatively free from fallen and broken stalks. In almost every case where it has been tested, the yield from this strain has been greater than the yield from the local variety planted in the same field.

SUMMARY

The results of 90 tests conducted over a period of four years seem to indicate the following:

1. The yield from seed corn on cobs free from internal discoloration is greater than the yield from seed corn on cobs with internal discoloration, provided other conditions are similar.
2. This difference in yield is greater on poorer corn land than it is on good corn land.
3. The differences in yield vary with the intensity of internal cob-discoloration; the slighter the internal discoloration, the greater the yield.
4. Internal cob-discoloration can be decreased by selection. It has been eliminated almost entirely in four years, by planting each year seed of ears free from internal cob-discoloration.
5. Corn grown from ears with the least internal cob-discoloration seems to have a more uniform stand, a smaller number of fallen and broken stalks, and fewer prematurely killed plants than corn grown from ears with the greatest amount of internal cob-discoloration.

UNIVERSITY OF MARYLAND,
COLLEGE PARK, MARYLAND.

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BLOSSOM BLIGHT OF THE PEACH

JOHN W. ROBERTS AND JOHN C. DUNEGAN

The brown rot disease of the peach caused by *Sclerotinia cinerea* (Bon.) Schröt, is, as its name suggests, best known as a rot which occurs on the ripe or nearly ripe fruit. There are, however, other manifestations of the disease, of which the most prominent is the form known as blossom blight, readily recognized by a withering of the blossoms and the production of conidial tufts on the diseased floral parts.

In central Georgia, blossom blight frequently causes severe damage, not so much by reducing the set of fruit as by establishing numerous sources of infection in the orchard, which later make it extremely difficult to prevent the fruit from rotting. Since the blighted blossoms frequently adhere to the twigs until after the fruit ripens, producing immense numbers of conidia during periods of rainy weather, they become a direct menace to the maturing fruit.

In other regions, such as parts of Delaware and the eastern shore of Maryland, the blighted blossoms are often so numerous as to curtail appreciably the size of the crop; but, under Georgia conditions, direct loss from this phase of the trouble is of infrequent occurrence.

That *S. cinerea* can produce blossom blight on peach trees has been known for many years. Smith (10, 11), in 1889, was among the first to mention the destruction of peach blossoms by this fungus. Since that time, Quaintance (8), Scott and Ayres (9), Jehle (4), McCubbin (6), Cook (2), McClintock (5), and many others have discussed blossom-blight of the peach, both as a destroyer of blossoms and in relation to the subsequent infection of the twigs and fruit.

Previous to Norton's (7) demonstration of the existence of the ascogenous stage of the fungus it was generally believed that infection of blossoms was due to conidia, although Smith (11) had pointed out that it was hard to understand how there could be a severe outbreak of blossom blight after a year in which there had been no brown rot of the fruit and no twig blight to transmit the disease. Since the publication of Norton's investigations, it has been generally assumed that ascospores are the principal source of infection of the blossoms, because they are usually produced in large numbers during the flowering period of the peach. Cook (2), however, reports severe cases of blossom-blight in orchards practically free from mummied fruit. He considers the infections to be related to the presence of cankers on the twigs. Scott and Ayres (9) state: "These ascospores, as well as

the conidia, serve to infect the blossoms." McClintock (5) produced characteristic blight by spraying peach blossoms with water containing suspended conidia obtained from mummied fruits. The inoculated blossoms were then covered with paper bags. McClintock worked with trees growing out of doors, and apparently no controls were used.

Experiments also have been made to determine the stage at which peach blossoms are most subject to infection and the most susceptible parts of the flower. Jehle (4) states: "Infection seemed to begin at the calyx or shuck and spread from there into the stamens and pistil, accompanied by a withering and browning of the diseased portion. Sometimes only the shuck becomes diseased and this is then shed without further injury to the pistil, but usually the entire flower becomes infected . . ." McCubbin (6) observed infected calyces of peach blossoms. Barss (1), in a discussion of blossom blight of stone fruits, states: "Petals or calyx lobes and likewise the stigmas of the flower are very subject to infection. The first sign is a small brown spot on the petal, calyx lobe, or tip of the pistil. This discoloration spreads rapidly if the weather continues moist. The disease eventually kills the parts attacked and begins to invade the base of the flower, which then turns dark."

The experiments reported upon in this paper were made chiefly for the purpose of determining the susceptibility of blossoms to infection by conidia and ascospores and the relative susceptibility of the different parts of the flower. The experiments by the senior author were made at Washington, D. C., and those by the junior author at Fort Valley, Georgia.

EXPERIMENTS AT WASHINGTON, D. C.

For the experiments reported upon here, the apothecia of *S. cinerea* and the peach branches bearing blossoms were obtained from Arlington Farm, Virginia, in the spring of 1924. In all cases the conidia were from cultures grown on four per cent potato agar, the same physiologic form being used throughout. This particular physiologic form, obtained from a single ascospore produced in an apothecium from a mummified plum, grows very rapidly and produces conidia more readily than any other grown by the senior author. The ascospores and conidia respectively were applied to the blossoms in watery suspension by means of an atomizer, except that, in the experiments of April 16, additional ascospores were applied by exposing the blossoms to a cloud of them expelled directly from apothecia. The control branches were sprayed with distilled water only. In all experiments there were three lots, receiving different treatments and kept separate under individual bell jars:

Lot 1. Sprayed with ascospores suspended in distilled water.
Lot 2. Sprayed with conidia suspended in distilled water.
Lot 3. Control. Sprayed with distilled water.

In each experiment the branches were cut in the orchard and brought into the laboratory before the blossoms had opened. Inoculations were made in the laboratory when nearly all the blossoms had opened. It was believed that the chances of accidental infection would be much less than when the branches with opened blossoms were taken directly from the orchard. At any rate, as shown by the results, there was little or no accidental infection. The number of blossoms in each lot varied from 39 to 215.

For the first and second experiments begun on April 12, branches of peach seedlings were used. For the third and fourth experiments, begun on April 15 and 16 respectively, branches of the Carman variety, the blossoms of which are moderately susceptible to the blight, were used. The results of these experiments are shown in table 1.

TABLE 1.—*The results of inoculating blossoms of peach seedlings and blossoms of the variety Carman with Sclerotinia cinerea at Washington, D. C., in 1924*

Lot	Inoculum	Percentage of blossoms blighted after 4–5 days			
		Experiment 1 (seedling)	Experiment 2 (seedling)	Experiment 3 (Carman)	Experiment 4 (Carman)
1	Ascospores suspended in distilled water	10	0	93	88
2	Conidia suspended in distilled water	40	20	90	56
3	Control, distilled water	0	0	0	0

All the blighted blossoms bore an abundance of the conidia of *S. cinerea*. All the blossoms of lot 3 (the controls) in all the experiments remained normal to the end except for the falling of a few petals. In lots 1 and 2, only those which were well opened were attacked by the fungus. In experiments 1 and 2, the infections were mainly on the stamens and pistils, the anthers and stigmas being the first to show the blight and the first to show production of conidia. These parts of the young blossoms, being rather closely pressed together, retained for some time the water in which the conidia or ascospores were suspended. Opened anthers seemed very susceptible. In subsequent experiments, all parts appeared about equally susceptible, the sepals and petals as well as the stamens and pistils being

literally covered with conidia in the few days allotted to the experiments. The differences in the results of the earlier and the later experiments may have been purely accidental, but in the later experiments the blossoms were more widely opened and received the suspensions of ascospores and conidia more directly upon their inner surfaces.

On drying, blighted blossoms with uninfected peduncles fell off, but those with infected peduncles adhered tightly to the twigs as under natural conditions in the orchard.

The inoculation experiments show that at a moderate temperature (25° C.), and under conditions of extreme moisture, only a few days are required for the development of blossom blight. In 48 hours small brown spots began to appear on the various parts, and the formation of conidia followed very closely. Blossoms only partly opened were not easily infected, and those escaping infection after being inoculated were usually in this condition. The presence of conidia on the blighted parts made reisolation of the fungus seem a useless procedure, but it was performed for each lot inoculated.

EXPERIMENTS AT FORT VALLEY, GEORGIA

Ascospores of *S. cinerea* from fresh apothecia and peach branches bearing blossoms were obtained from orchards in the immediate vicinity of Fort Valley.

Conidia were obtained by flooding with sterile water young cultures of the fungus isolated from a peach and growing on potato dextrose agar. The conidia were collected by centrifuging the water suspension and were applied directly to the stigmas with a platinum loop, or the water suspension was sprayed on the blossoms with an atomizer.

Ascospores were secured by crushing the mature apothecia in sterile water. The ascospores were obtained from the crushed material in water by centrifuging and were applied directly to the stigmas with a platinum loop, or the water suspension was sprayed on the blossoms with an atomizer.

Sterile water was either applied with a platinum loop directly to the stigmas of the blossoms used as checks or was sprayed onto all parts.

The twigs bearing the inoculated blossoms and the checks were placed in individual moist chambers to provide conditions favorable to the development of the fungus.

The experiments of 1924 were conducted with the varieties Hiley and Uneeda. The blossoms were inoculated on the stigmas only. Typical blossom blight developed on both of these varieties following inoculation of the stigmas with either conidia or ascospores. The first signs appeared on the stigma, but infection progressed rapidly down the style and finally involved the whole blossom. The anthers and sepals seemed to be slightly more susceptible than the petals, but abundant spore tufts were finally

produced on all the floral parts. Occasionally the fungus continued downward through the peduncle and formed small cankers on the twigs. When this occurred, the blossoms adhered to the twigs as under natural conditions in the orchard.

The check blossoms remained normal in practically all cases, save for the dropping of the petals. The few that developed blight were believed to have received chance infections prior to their removal from the orchard.

Symptoms typical of blossom blight as found in the orchard were produced repeatedly as the result of stigma inoculations with conidia and ascospores. However, since the two kinds of spores may settle on any part of the blossom under natural conditions in the orchard, additional inoculations were made in 1925 by spraying all parts of the blossoms with a water suspension of the spores.

TABLE 2.—*The results of inoculating blossoms of the peach varieties, Carman and Uneeda, with Sclerotinia cinerea at Fort Valley, Georgia, in 1925*

Lot	Inoculum	Percentage of blossoms blighted after 5–6 days	
		Experiment 1 (Carman)	Experiment 2 (Uneeda)
1	Ascospores suspended in distilled water.	61	—
2	Conidia suspended in distilled water.	93	93
3	Control, distilled water.	0	0

The first experiment was started on March 9, and the second on March 14, 1925. For the first experiment, twigs of the variety Carman bearing open blossoms were selected from an orchard in which no apothecia could be found. For the second experiment, blossoms of Uneeda, a variety quite susceptible to the disease as it occurs in the orchard, were used. Twigs bearing open blossoms and numerous buds had been brought to the laboratory on the 10th and all the open blossoms removed. By March 14 a number of the buds had opened in the laboratory. Only conidia were used in this experiment, as apothecia with viable ascospores were no longer available.

For the first experiment, the twigs were divided into three lots and treated as follows:

Lot 1. Sprayed with a suspension of ascospores in distilled water.
Lot 2. Sprayed with a suspension of conidia in distilled water.
Lot 3. Control. Sprayed with distilled water.

In the second experiment lot 1 was of course omitted. The number of blossoms in each lot varied from 88 to 129.

The twigs standing in jars of water were placed in individual iceless refrigerators as devised by Hunt (3). The results of these experiments are shown in table 2.

The stamens, anthers and sepals were in most cases covered with conidial tufts. The petals and stigmas, while they were quite susceptible, did not seem to serve as a locus for conidial production as readily as the other parts. The anthers were particularly susceptible.

CONCLUSIONS

The inoculation experiments reported here indicate that both ascospores and conidia can infect any part of the peach flower, each possessing about the same degree of pathogenicity. As either or both may be present when peaches are in blossom, they are both to be regarded as dangerous, and pathologists should consider the prevalence of both in relation to the disease.

SUMMARY

Inoculation experiments carried on at Washington, D. C., and Fort Valley, Georgia, show that the conidia and the ascospores of *Sclerotinia cinerea* (Bon.) Schröt. are capable of causing a blossom blight of the peach.

All parts of the open blossoms may be attacked. Direct infections of the stigmas, anthers, petals, and inner surfaces of the sepals were observed.

It is concluded that either ascospores or conidia, or both, may be responsible for outbreaks of blossom blight in peach orchards.

OFFICE OF FRUIT DISEASE INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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THE DISTRIBUTION OF CRANBERRY FALSE BLOSSOM

NEIL E. STEVENS AND W. H. SAWYER, JR.

The economic importance of "false blossom" of the cranberry (*Vaccinium macrocarpum*) has made necessary a careful field study of the disease for the purpose of discovering possible resistant varieties, or conditions of culture under which the severity of the disease had been reduced. This survey has yielded some interesting additions to our knowledge of the present distribution and manner of spread of the disease. This paper summarizes the available knowledge of the distribution of false blossom and contains information obtained since the last publication on the subject, to which reference may be made for citations of the literature on this disease.[1]

The cause of false blossom has not been determined. Most of those who are now actively studying cranberry disease problems in the field, however, have come to agree that it behaves like an infectious disease; and the present writers are much more concerned with its control than with its cause.

THE DISTRIBUTION OF FALSE BLOSSOM IN NEW ENGLAND

In the Cape Cod region of Massachusetts, false blossom is now known to occur on 52 bogs. The diseased bogs are largely in Plymouth County. On at least six of them the disease first was found on vines brought from Wisconsin. A surprising number of the remaining bogs which now show false blossom are known to have been planted, at least in part, with vines from bogs on which the disease had already become established. Many of these originally diseased bogs contained at one time either Wisconsin vines or vines of the Massachusetts variety, variously known as Holliston, Bachelor, Mammoth, and Small's Number One. The last-named variety seems to be very susceptible to false blossom and is generally diseased wherever found in Plymouth County. No false blossom could be found, however, in the original plantings of this variety in the town of Holliston, which led Dr. Franklin[2] to suggest that at least some of the vines which are called Hollistons on Cape Cod might really be Bennetts, a Wisconsin variety. Support is given to this theory by the fact that the two varieties are so similar in appearance that we are not yet able to distinguish them, and by the fact that Emulous Small, who was one of the most active Cape Cod

[1] Stevens, Neil E. Field observations on false blossom of the cultivated cranberry. Phytopath. **15**: 85–91. 1925.

[2] Franklin, H. J. Report of the cranberry station for 1919 and 1920. Mass. Agr. Exp. Sta., Bul. 206: 149–168. 1921.

growers in introducing and distributing Holliston vines, was a life member of the Wisconsin Cranberry Growers' Association and an intimate friend of at least one Wisconsin grower. To present in detail the information which has been accumulated on the spread of this disease from bog to bog in Massachusetts would occupy too much space. One or two examples may, however, be given.

Outside Plymouth County, a severe infection of false blossom on the Howes variety was found recently by Dr. Franklin on a bog near New Bedford, in Bristol County. The vines for planting this bog came from a diseased bog near East Wareham, Mass. In Chatham, Barnstable County, Dr. Franklin also found scattered vines affected with false blossom on one bog planted with a local variety, Smiths, and with Howes; but a part of the Howes proved to have come from a small bog which in turn had been planted from a diseased bog in Carver. Careful search on other bogs in the neighborhood, containing the Smith variety, as well as other varieties, failed to show any false blossom. As yet false blossom has not been found on any of the small bogs of the "lower cape" (Barnstable County) which are planted wholly to local varieties.

Genuine "wild bogs" are now rare in the Cape Cod region, but they are fairly common in Maine and New Hampshire. Careful search on many of these wild bogs, whenever opportunity offered during the past five or six years, has failed to show any false blossom on any wild bog in this region. This is in striking contrast to the distribution of the fungous diseases of the cranberry. Many of these diseases are very widely distributed on wild bogs. Such fruit-rot fungi as *Fusicoccum putrefaciens* Shear, for example, have been found in cranberries gathered on wild bogs in northern Maine, and in mountain cranberries (*V. vitis-ideae*) from several remote localities. Rose bloom (*Exobasidium oxycocci* Rost) is also widely distributed on cranberries throughout this region and was found several years ago by Dr. Shear on a tiny patch of *V. oxycoccus* near Lake of the Clouds, only a little over a mile from the summit of Mount Washington in New Hampshire. False blossom has not yet been found on cranberries in either New Brunswick or Nova Scotia, Canada, although there are cultivated bogs in the latter province. The absence of false blossom on wild bogs in New England and the adjacent parts of Canada, together with the fact that in many cases the infection is known to have come from bogs already diseased, suggests that false blossom is not native in the region but was introduced from Wisconsin.

DISTRIBUTION OF FALSE BLOSSOM IN NEW JERSEY

Our knowledge of the occurrence of false blossom is less complete in New Jersey than in New England. Through the generous assistance of Mr. C.

S. Beckwith, of the New Jersey Experiment Station, one of the writers has been able to make a fairly satisfactory study of bogs in different regions of this State, and the information thus obtained seems to justify the following statement concerning conditions in New Jersey. False blossom is widely distributed in New Jersey, though only in a very few cases has it become markedly destructive. It was found on almost every bog containing Howes, the most commonly planted variety which comes from outside the state. Unlike Cape Cod, New Jersey has large areas of native, more or less mixed vines, some of which are cultivated and some wild. False blossom is very rare on such bogs. In fact, although "Native Jerseys" have proved very susceptible to false blossom on certain bogs where they were grown close to infected Howes or Hollistons, false blossom has not yet been found on any bog containing only Native Jerseys.

On at least three bogs on which false blossom now exists, vines from diseased bogs are known to have been planted. In one case the diseased vines had come from Wisconsin, and in two cases from Massachusetts. The fact that false blossom is widely distributed in New Jersey on vines of Massachusetts or Wisconsin varieties, but is unknown on "Jersey" vines except where these are planted near introduced varieties, seems best accounted for by the supposition that the disease is not native in New Jersey but was introduced both from Massachusetts and Wisconsin.

THE FALSE BLOSSOM SITUATION IN WISCONSIN

In the course of a survey of false blossom distribution in Wisconsin during the summer of 1925, the disease was found on 48 of the 61 cultivated bogs[3] examined. Of these, at least 20 have infections severe enough to materially affect the crop. It probably occurs on other bogs where it was not found.

False blossom is believed by some of the older growers to have been present in the State at least as early as 1885. It did not, however, receive serious attention as a disease until many years later. About 1900 a considerable planting was made in the Mather district of three varieties of vines from the Berlin region, namely, Palmeter, Bell and Bugle, and Metallic Bell. By 1906 false blossom had become prevalent in these varieties, and since then it has become so severe that their cultivation has been materially reduced. Contrary to the general belief that false blossom is on the wane in Wisconsin, a careful comparison of the same varieties on the same bogs over a period of five years seems to show an increase in the amount of disease in 1925 over previous years. The apparent decline of the disease may well be due, not

[3] Cultivated areas of cranberries, which in other cranberry sections are known as *bogs*, are in Wisconsin generally referred to as *marshes*. We are here using the shorter word for the sake of uniformity.

to an actual decrease of false blossom on individual bogs, but to the fact that the most susceptible varieties are being replaced by those which are more resistant.

The severity of false blossom in the Mather district on varieties brought from near Berlin, Wisconsin, has given rise to the general belief that it was introduced into the Mather region on the Berlin vines. It is perhaps even more probable that the disease was already present in or near Mather and that the Berlin vines proved particularly susceptible. The observations in 1925 show at least that false blossom is less common in the Berlin region than in any other cranberry district of Wisconsin. It is probably significant that false blossom is more widely distributed in Wisconsin than in any other cranberry-growing state. Even more significant is the occurrence in Wisconsin of this disease on wild cranberry vines remote from cultivated bogs.

FALSE BLOSSOM ON WILD BOGS IN WISCONSIN

Wild cranberry vines are now very few in the Mather and Cranmoor regions apparently owing to the fact that drainage operations which have dried out the bogs have been followed by fires which destroyed the vines. In the northern part of the State, however, wild cranberry bogs may still be found. The time available for field work permitted the examination of but few of these wild bogs. Nevertheless, on two of them northwest of Spooner, false blossom was found in abundance. One is three miles and the other eight miles from a cultivated bog, and both are over two hundred miles from the region where false blossom is abundant on cultivated bogs. Indeed the disease was introduced into the nearest cultivated bog by diseased plants taken from one of these wild bogs. It seems impossible, then, for the disease to have been introduced into these wild bogs from cultivated ones. The only reasonable assumption is that it is indigenous.

CONDITIONS ON THE PACIFIC COAST

In Oregon, where the cranberry plantings were made largely with Wisconsin vines, false blossom is present on practically all the bogs. It is not serious, however, in this region. Mr. H. F. Bain, who has been studying cranberry diseases in Oregon and Washington for the last four years, is of the opinion that false blossom does not spread, or at least spreads very slowly, in that region; while Dr. Shear, who examined most of the Oregon bogs for false blossom in 1919 and again in 1924, reports a decrease during that period in the amount of the disease on the bogs observed. In Washington, false blossom is known to occur on only one bog. This was planted with vines from Wisconsin. The absence of false blossom on other bogs in Washington may be due in part to the fact that the disease seems to spread

very slowly in the Columbia River region, and also to the fact that a large part of the vines are McFarlins, a variety resistant to false blossom.

VARIETAL RESISTANCE TO FALSE BLOSSOM

As previously reported, there is no generally grown variety of cranberries which is free from false blossom. There is, however, a difference in the severity of the disease on different varieties. In Wisconsin, Palmeter, Bell and Bugle, Metallic Bell, Bennet Jumbo, Prolific (a Michigan variety), and the Mammoth (a Massachusetts variety, there called Holliston) have proved generally susceptible. The Searls is usually free from false blossom in the Cranmoor district. In the Mather region, however, and near Minong, Wisconsin, there are some very severe infections on this variety. The Searls has proved very susceptible to false blossom also when planted in Massachusetts. The McFarlin, however, while occasionally attacked by false blossom, has proved the most highly resistant of any variety, both in Massachusetts and Wisconsin.

CONTROL

At present, the most promising control method seems to be the regular spring and fall flooding of the bogs combined with general good culture. With this should go the elimination of the most highly susceptible varieties, and the planting of McFarlins in badly diseased areas on bogs where experience has demonstrated that this variety can be grown to advantage.

A promising means of control which is now being tried in Wisconsin, and the progress of which will be followed with interest, is the practice of holding the winter flowage very late (about July 1) for a year or two.

SUMMARY

"False blossom" of the cranberry has been found in Wisconsin on wild vines remote from cultivated bogs. It has not yet been found on wild vines in any other cranberry-growing region. The disease is also more generally distributed on cultivated cranberries in Wisconsin than in any other State.

The distribution of false blossom in New England and on the Pacific Coast seems to indicate that it was introduced into those regions by means of diseased vines brought from Wisconsin.

The disease apparently was introduced into New Jersey on diseased vines both from Massachusetts and Wisconsin.

The information now available suggests that false blossom first occurred in the United States on cranberries in Wisconsin and from there was spread to other cranberry-growing regions in shipments of diseased vines.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

RELATION OF STRAWBERRY FRUIT ROTS TO WEATHER CONDITIONS IN THE FIELD

DEAN H. ROSE

In an earlier paper[1] the writer presented the results of a two years' study of leather rot of strawberry fruits caused by a fungus apparently identical with *Phytophthora cactorum* Leb. et Cohn. The chief conclusion therein drawn, aside from that relative to the cause of the disease, was "that there is a close relation between leather rot and rainfall and that, in the region studied, the rot can be expected to reach a maximum within three to four days after heavy rains." The further statement was made that "temperature is important also, but its effect can not be determined without further study."

These conclusions were based on work done chiefly at Beebe, Arkansas, during the season of 1923. It is worth reporting, therefore, how they agree with those to be drawn from work done (also at Beebe, Ark.) during the picking season of 1924, especially since it has not been possible to continue the work in 1925.

Results obtained during 1923 and 1924, together with temperature and rainfall records, are summarized in tables 1 and 2, and shown graphically in figure 1. Weather notes are given in table 3.

The methods used during the second year's work were the same as those

TABLE 1.—*Percentages of leather rot, other rots, and total rot in strawberries used in holding tests, on first day after removal from storage, Beebe, Ark., 1924*

Kind of rot	Dates of picking; May, 1924														
	7	8	9	12	14	17	19	21	23	24	26	27	28	29	30
	Percentage of Rot														
Leather rot	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.0	0.7	0.5	1.9	0.3
Other rots	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.6	2.7	2.3	10.6	1.2
Total rot	0.7	0.9	0.0	0.8	0.4	0.2	0.4	0.0	0.1	1.8	3.6	3.4	2.8	12.5	1.5

[1] Rose, Dean H. Leather rot of strawberries. Jour. Agr. Res. 28: 357–375. 1924.

described in the earlier paper. Berries from the various experimental plots or from other fields around Beebe were held in the local cold storage plant for four days, then taken out, hand sorted, and counts made to determine the number of sound berries, the kinds of rot present, and the percentage of each. Since the percentages were not large at any time, and there were no observable differences among the various plots, the data from the experimental field are reported for the field as a whole and not for each plot separately. As in the earlier report, the percentage of rot is recorded (Table 1 and Fig. 1) for the day on which the picking was made, although it was not determined until four days later. Berries used from May 7 to 23 were of the Klondike variety; those used from May 24 to 30 were of the Aroma variety.

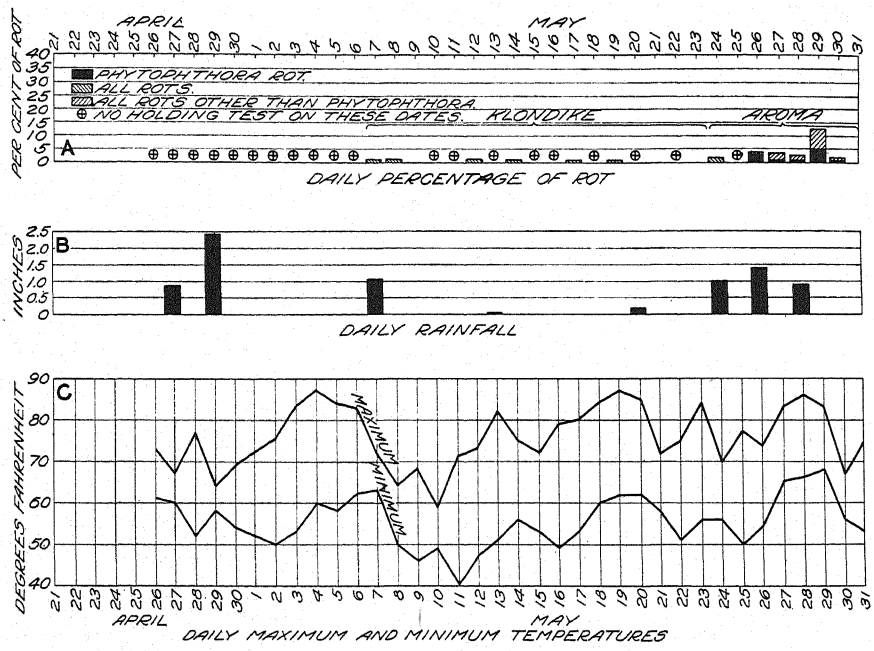

FIG. 1. A. Percentage of rot found, immediately after removal from storage, in various lots of strawberries held for four days at a temperature of 40–45° F. Percentages are entered for the day on which the berries were picked. B. Daily rainfall in inches. C. Daily maximum and minimum temperatures recorded at Beebe, Arkansas, by means of standard apparatus loaned by the United States Weather Bureau. Period covered, April 26 to May 31, 1924.

In 1924 there was almost no rot of any sort except during the last few days of May, when leather rot and other rots, chiefly Rhizoctonia rot, began

to appear in the field and in the packed fruit; even on these dates the percentages were very much lower than those reported for a large part of the 1923 season. It will be noted, however, that in 1923 the rainy periods during the picking season were followed by warm weather, while in 1924 all of the rains, with the exception of those on May 24 and 26, were followed by relatively cool weather. Therefore the inference seems justified that, in the region studied, rot of strawberries in the field (enlarging the earlier statement so as to cover leather rot and all other rots) is dependent upon two factors, rainfall and temperature. If rains are followed by cool weather, there is less danger of rot; if followed by warm weather, rot is likely to be serious. This conclusion is practically that arrived at on the basis of the earlier work, except that it includes a more definite and better documented statement on the relation of temperature to the incidence of rot. On one point the data for 1924 are less clear than those for 1923.

TABLE 2.—*Daily temperatures and rainfall, April 26 to May 31, 1924, at Beebe, Ark.*

Date	Temperature in degrees F.		Rainfall in inches	Date	Temperature in degrees F.		Rainfall in inches
	Maximum	Minimum			Maximum	Minimum	
1924							
April 26	73	61	0	May 14	75	56	0
27	67	60	.85	15	72	53	0
28	77	52	0	16	79	49	0
29	64	58	2.49	17	80	53	0
30	67	52	0	18	84	60	0
May 1	72	50	0	19	87	62	0
2	75	48	0	20	85	62	1.45
3	83	51	0	21	70	58	0
4	87	60	0	22	75	51	0
5	84	58	0	23	84	56	0
6	83	62	0	24	70	56	.9
7	72	63	.53	25	77	50	0
8	64	50	0	26	74	54	1.39
9	68	46	0	27	83	65	0
10	59	49	0	28	86	66	.88
11	71	40	0	29	83	68	0
12	73	47	0	30	67	56	0
13	82	51	0	31	74	53	0

There seems to be a lag in the appearance of rot following the rains late in May, but it is not so clearly defined as during the period May 14–18, 1923.

At no time in 1924 was leather rot as serious, compared with other rots, as it had been in 1923.

TABLE 3.—*Weather notes, Beebe, Ark., from April 26 to May 31, inclusive, 1924.*

April	26—Cloudy	May	14—Clear
	27—Cloudy and rain		15—Partly cloudy
	28—Cloudy		16—Partly cloudy
May	1—Clear		17—Hazy
	2—Clear		18—Clear
	3—Clear		19—Clear
	4—Clear		20—Cloudy
	5—Clear		21—Cloudy
	6—Cloudy		22—Clear
	7—Rain		23—Cloudy
	8—Cloudy		24—Partly cloudy
	9—Cloudy		25—Clear
	10—Cloudy		26—Cloudy
	11—Clear		27—Clear
	12—Partly cloudy		28—Cloudy
	13—Cloudy		29—Partly cloudy
			30—Clear
			31—Cloudy a. m.

OFFICE OF FRUIT DISEASE INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

International Problems in Plant Pathology: An Invitation.—The forthcoming International Congress of Plant Sciences (Fourth International Botanical Congress) is to be held at Cornell University, Ithaca, New York, U. S. A., from August 16 to 23, 1926. Invitations are being issued to all plant workers in the world to attend. The Congress will be divided into about twelve separate sections for the presentation of papers and the transaction of business of interest to the section. Plant Pathologists (the word is used in the restricted, or American sense) will organize in Section J. A formal program of invitation papers is being arranged. Ample time will be reserved for round-table discussions, field trips, exhibitions, etc.

Although it has been decided that no international legislation will be enacted at this congress, there will be opportunity in Section J (and doubtless in other sections as well) for the presentation of "proposals" for the consideration of the Section. Any person who wishes to make a proposal relative to any phase of plant pathology is invited to observe the following points:

The proposal should deal with a problem of international significance; it should be brief and concise; an oral or written argument should be prepared in advance; an abstract of not to exceed 500 words should be submitted to the Secretary not later than May 1, 1926. Proposals may be admitted to the program after May 1, only on condition that the author prepare an abstract in replica of about 100 copies in at least three languages.

The secretary for Section J is Donald Reddick, College of Agriculture, Ithaca, New York, U. S. A., to whom all communications regarding the business of the Section should be addressed.

A Simple Method of Inoculating Potatoes with the Spindle-tuber Disease.—A simple modification of the tuber graft method, by which an average of 90 per cent infection has regularly been obtained with spindle-tuber, has been found very advantageous. The same method also has been used with mosaic and leaf roll. A cork borer of 8–10 mm. in diameter is used to bore a hole through a healthy seed piece, and the core of tissue from an infected tuber obtained with a slightly larger cork borer is forced into the hole. Perfect contact of a relatively large surface is thus obtained, and inoculations can be made rapidly, three inoculated and one check plant usually being obtained from a healthy tuber. Double inoculations also can be made easily. The disease appears early, and the new tubers have distinct symptoms.—R. W. Goss, Nebraska Agricultural Experiment Station, Lincoln, Neb.

Verticillium Wilt of Tomatoes in California.—In the March, 1925, number of PHYTOPATHOLOGY, Mary K. Bryan called attention to a wilt disease of tomatoes which occurs in northern Ohio and Erie County, Pa., and which she has proved to be due to a *Verticillium*—probably *V. albo-atrum.* Hers is the only reference in the literature of America to a *Verticillium* wilt disease of tomatoes. She expressed the opinion that in regions where cool temperatures prevail this organism is responsible for much of the damage now attributed to *Fusarium vasinfectum,* since the symptoms of the wilts produced by the two organisms are identical.

In the San Francisco Bay region, California, a very serious wilt disease of tomatoes has been found to be due to a species of *Verticillium* resembling *V. albo-atrum.* In October, 1924, this organism alone was found associated with wilted plants in tomato fields in which as many as 90 per cent of the plants were destroyed. The fungus was isolated and its pathenogenicity proved by successful inoculations on tomatoes in the greenhouse.

The fungus has been found to be identical with the *Verticillium* which causes the so-called "black heart" of stone fruits, a widespread disease of considerable importance described in California by Czarnecki.[1]

It is also identical with the *Verticillium* which causes "blue stem" of bush fruits there, the Ranaree raspberry being particularly susceptible to attack.

Single spore cultures of the *Verticillium* isolated from typical cases of blue stem of raspberry, black heart of apricot and peach, and wilt of tomatoes have been used to inoculate tomatoes. The fungus from each of these sources attacked tomato plants with equal readiness, and typical wilt symptoms were produced. The fungus was eventually recovered by the usual methods from a large number of plants used in the experiments. Passage of the fungus from these same sources through Myrobalan plum seedlings and young Ranaree raspberry plants has been accomplished. Typical wilt symptoms manifested by these plants were produced, and the fungus was recovered in a number of instances.

Further discussion of the *Verticillium* wilt diseases of these economic plants in California is reserved for a later paper.—B. A. RUDOLPH, University of California Deciduous Fruit Station, San Jose, Cal.

Berberis fendleri, an Alternate Host of Puccinia graminis tritici.—*Berberis fendleri* Gray, the wild barberry, native to certain sections of the Rocky Mountains, for some time has been considered a host of two grass rusts. Arthur,[2] in North American Flora, reports this species of barberry not only

[1] Czarnecki, H. Studies on the so-called black heart disease of apricots. Phytopath. 13: 216–224. 1923.

[2] Arthur, J. C. N. Amer. Flora 7: 295–330. 1920.

as a host of *Puccinia montanensis,* but also lists it as an alternate host of *P. graminis.* However, no reported inoculation experiments give proof of this relationship, although Stakman and Levine[3] found *B. fendleri* rusted with *P. graminis* at Highland Park, Rochester, New York. Mains[4] indicated some discrepancies in the relation of *B. fendleri* to *P. montanensis* which suggest a confusion with *P. graminis.*

As *B. fendleri* is prevalent in sections of Colorado not far removed from cultivated areas, it seemed worth while to test its susceptibility to *P. graminis tritici* under controlled conditions. This led to the inoculation of young, rapidly growing shoots of *B. fendleri* with sporidia of *P. graminis tritici.* These sporidia were obtained from germinating teliospores borne on *Hordeum jubatum* growing some 200 miles from any *B. fendleri.* The teliospores were germinated during the winter of 1924–25, and the sporidia sown on the *B. fendleri* shoots in the greenhouse. After inoculation, the plants were kept in a moist chamber for three days and then transferred to muslin-covered booths. Within seven days from the time of inoculation, profuse pycnial development was manifest, and in 21 days normal aecia developed in large numbers on the leaves. The infection was so extensive that many of the leaves were killed. The character of the aecia and the spore measurements are those of *P. graminis.* Parallel inoculations on *B. vulgaris* were equally successful.

Inoculations on Little Club wheat with the aeciospores produced profuse uredinial infection in eight days. The uredinia were large and confluent, and the spores were of the same size, shape, and pore arrangement as in *P. graminis.* No paraphyses were noted.

This test, successively repeated, definitely places *B. fendleri* among the susceptible species of the genus *Berberis,* and indicates beyond doubt that this shrub can act as an alternate host for *P. graminis tritici.*—L. W. DURRELL, Colorado Agricultural College, and E. A. LUNGREN, Office of Cereal Investigations, U. S. Dept. Agr.

Similarity of Bacterial Diseases of Avocado, Lilac, and Citrus in California.—During the spring of 1925, a new bacterial blemish on the fruit of avocado (Guatemalan type) and a bacterial disease of the lilac were studied. These new diseases are apparently caused by *Pseudomonas citriputeale,* which causes the Citrus black pit and blast. This organism was first described as causing a depressed spot on lemons.[5] Later, Citrus blast was

[3] Stakman, E. C., and M. N. Levine. A partial report on the susceptibility and resistance of *Berberis* and related genera to stem rust. Cereal Courier **15**: 278–287. 1923.

[4] Mains, E. B. The heteroecism of *Puccinia montanensis, P. koeleriae,* and *P. apocrypta.* Mycologia **13**: 315–322. 1921.

[5] Smith, C. O. Black pit of lemons. Phytopath. **3**: 277–283. 1913.

described and the causal organism isolated.[6] Finally the two troubles were again studied[7] and found to be two manifestations of the same organism. The spots on the fruit of avocado are brownish to black, firm, irregular to circular, 2–10 millimeters or larger upon coalescing. They do not extend far into the flesh but are limited to the rind. The lenticels are the channels of infection. The California lilac disease forms a circular spot 2–5 millimeters on the leaves, and a definite lesion on the stem. The causal organisms from both have been isolated, and their cultural characteristics and pathogenicity on different hosts are being studied, as well as their relationship to the European lilac disease caused by *Pseudomonas syringae*.[8] This investigation is well in progress, and the results thus far indicate that the Citrus blast, the avocado blemish, and the California lilac disease are closely related and probably are caused by the same organism.—CLAYTON O. SMITH, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

Holcus Bacterial Spot on Species of Holcus and Zea mays.—Bacterial leaf spots have been known on species of *Holcus* and *Zea mays* L. since Burrill's early, epoch-making work on bacterial diseases of plants. There has been, however, much confusion as to the identity of the causal agent or agents. A study of these bacterial spots has permitted a much clearer understanding of the cause and has definitely associated with the disease a single species of *Bacterium* which is quite cosmopolitan in its host range.

The symptoms of the Holcus bacterial spot vary somewhat on different hosts, a fact which doubtless has been responsible for much of the confusion in the earlier writings. However the lesions on all hosts are round, oblong, linear to irregular, and of varying size. On sorghum (*Holcus sorghum* L.), sudan grass (*H. sorghum* var. *sudanensis* (Piper) Hitchc.), and Johnson grass (*H. halepensis* L.), the spots are red or light centered with a red border, except on the variety of *H. sorghum* known as Shallu, on which the spots have a dark brown border. The lesions on pearl millet (*Pennisetum glaucum* (L.) R. Br.) are dark brown with a slight halo, while on corn (*Zea mays*) they are light to dark brown with a reddish brown border and a light green halo when viewed by transmitted light. The spots on foxtail (*Chaetochloa lutescens* (Weigel) Stuntz) are small and dark brown.

[6] Lee, H. A. A new bacterial disease of Citrus. Jour. Agr. Res. **9**: 1–8. 1917.

[7] Fawcett, H. S., W. T. Horne, and A. F. Camp. Citrus blast and black pit. Calif. Agr. Exp. Sta., Tech. Paper 5. 1923.

[8] Van Hall, C. J. J. De Seringenziekte, veroozaakt door *Pseudomonas syringae*. Bijdragen tot de kennis der bakterieele plantenziekten: 141–146. Amsterdam, 1902.

A white, fluorescent bacterium has been consistently isolated from the necrotic areas on the different hosts, and numerous cross-inoculations and cultural studies have shown that the organisms from the various hosts are identical. A careful comparison of the causal organism with previously described bacterial organisms on these hosts, as well as closely related hosts, indicates that this disease is caused by an undescribed *Bacterium*. The organism is briefly characterized as follows:

Bacterium holci n. sp.[9]

Short cylindrical rods, rounded at the ends, solitary or in pairs; average size .73μ by 2.13μ; motile by 1 to 4 polar flagella at one pole only; no spores, involution forms, or capsules; strictly aërobic; stains readily with gentian violet or carbol fuchsin; gram-negative.

Surface colonies on beef-peptone agar are round, raised, smooth, entire margin, finely amorphous; grayish white in reflected light, slightly greenish fluorescent in transmitted light.

Gelatin rapidly liquefied; nitrates reduced; no indol; no gas from various carbohydrates; acid from dextrose and saccharose; no acid in milk; casein digested without curdling; growth and greenish pigment formation in Uschinsky's and Fermi's solution; no growth in Cohn's solution; tolerates six per cent sodium chloride in pH 7 beef-peptone broth; grows in pH 5.0, but not in pH 4.5 beef-peptone broth.

Temperature growth range from 0° to 35° C., optimum between 25° and 30° C.; thermal death point, 49° C.; resistant to freezing in water; susceptible to desiccation on glass, but resistant on *Holcus sorghum* seed. Killed by 45-minute exposure to direct sunlight.

Group number 211.2323133.

Pathogenic on *Holcus sorghum* L., *H. sorghum* var. *sudanensis*, (Piper) Hitchc., *H. halepensis* L., *Pennisetum glaucum* (L.) R. Br., *Zea mays* L., *Z. mays* var. *saccharata* Bailey, and *Chaetochloa lutescens* (Weigel) Stuntz.—JAMES B. KENDRICK, Plant Pathological Laboratories, Iowa State College, Ames, Iowa.

The 1925 Summer Field Meeting—Cereal Disease Conference. The 1925 cereal disease field meets and conferences took place this year in three different States, Iowa, Minnesota, and North Dakota, starting July 8 at Ames and closing July 14 at Fargo. The meeting was authorized and held under the direction of the Advisory Board and under the immediate supervision of a general committee. Local committees for each state arranged

[9] According to Migula's classification and the revision adopted by the committee of the Society of American Bacteriologists, the combination would be *Pseudomonas holci* n. sp. Winslow, C.-E. A. et al. The families and genera of the bacteria. Jour. Bact. 5: 191–229. 1920.

programs to provide opportunity for a study of conditions and methods of disease control in operation for each of the major crops, including garden vegetables and truck crops.

The total attendance registered at the three meetings was 105. The registration at Ames was 42, at St. Paul 74, at Fargo 43. Of those in attendance at Ames, 30 went on to Minnesota. Of those at the Minnesota conference, 25 continued through to North Dakota. Twenty attended the meetings and field work in each of the three States, including representatives from the Department of Agriculture, Washington, D. C., from Ohio, Indiana, Wisconsin, Minnesota, Iowa, Kansas, New York, Delaware, North Dakota, Montana, New Mexico, and Hungary.

The rust and smut problems naturally attracted the greater number of workers. The barberry eradication forces were well represented by Dr. F. E. Kempton and the state leaders from Indiana, Ohio, Minnesota, Montana, Wisconsin, Iowa, and North Dakota. Within each of the states this number was increased by the attendance of various district workers. The Divisions of Agronomy at each of the institutions cooperated very closely with the pathologists and were particularly helpful in explaining to the visiting delegates the various field experiments under way. At Ames a full day was given to the study of the work in the laboratories, greenhouses, and the field plots on the separate farms. The first half-day at St. Paul and at Fargo was given over to a study of the experimental plots, and the remaining day and one-half to trips into the surrounding farming country. At Ames, attention was given to experiments on seed treatment of sweet corn, to studies on crown gall, the cereal disease gardens, plots for breeding oats for resistance to crown rust, treatment methods with smuts, studies in cedar apple rust, cabbage yellows, potato seed treatment, field corn seed treatments, fertilizer experiments, and numerous studies in the vegetable gardens. At St. Paul the field and agronomy plots on the University Farm were inspected, including the disease control experiments and plant breeding nurseries. At Fargo the seed standardization gardens for potatoes, alfalfa, wheat and flax, the flax and wheat disease control gardens, agronomy plots in cereal breeding, crop rotation areas, and fertilizer areas were studied.

At each of the institutions the work was started by an address of welcome from the President. At Ames, Drs. Humphrey and Stakman gave very interesting outlines on the high lights of plant disease control as accomplished during the past two decades. At Minnesota, all speech making was eliminated from the evening program. The lady members of the Division of Plant Pathology put on an interesting tableau-like pageant, "The Dance of the Rust Spores." Through posing, singing, and dancing, the young women, aided by an artful arrangement of specially painted scenery repre-

senting the wheat crop and the barberry bushes, and through skillful manipulation of properly colored toy balloons, portrayed the life history of the black stem rust. This was followed by a moving picture outlining the details of the life history of the rust plant in a splendid educational way. At Fargo at the evening dinner a number of speakers were called upon to outline the status of their chief investigations. Each of the evening meetings was a splendid exhibition of the proper mixing of pleasure with the real purposes of cooperative education. At different times, sometimes in the field, sometimes on the lawns at noon, splendid expositions of the present status of their investigations were outlined by Drs. Dietz, Mains, Tisdale, Levine, Husz, and others. At the closing session at Amenia, North Dakota, Dr. F. E. Kempton gave an interesting survey of the present status of barberry eradication in the thirteen States in which the work is now being pushed.

These cereal field meets and conferences have very greatly aided in bringing together the investigators and emphasizing the importance of cooperative effort in the different States. An outstanding feature of this meeting was the effort on the part of each investigator to make clear the work as laid out in his experiments and as shown under the local conditions, so that the visitors working on similar problems could not fail to gain much valuable information. The field tours into the farming and land development districts of the three States also furnished splendid opportunity to see wherein the results of past investigations were being put into operation. There were places in each of the States wherein it was evident that no attention whatever had been given to such matters by the average grower, and there were other communities in which it was apparent that the work done by plant pathologists in the past decades had taken effect in practical agriculture. This was particularly marked in the work of the larger land development projects, under the management of skilled or trained foremen.

In each State over 200 miles of travel was made through the very best cereal districts. The workers had opportunity to get into the field at any point desired in addition to those outlined in the local programs. These drives gave opportunity to notice the astonishing effect of the present barberry eradication work in Iowa, Minnesota and North Dakota. It was clear that something had been done to stem rust of oats, wheat and barley. Great districts were found in each of the three States in which it was difficult for the experts to locate anything like destructive forms of infection. There were other areas visited in which the rust infections were astonishingly severe, quite discouraging to those who had expected too early results from the eradication accomplished to date.

Another very outstanding feature is the splendid line of investigations looking toward the establishment of accurate fundamental methods of breeding for disease control. The accomplishments in each of the States, particularly on the experimental grounds, must certainly have been very enlightening to the best informed workers in the group. No one of these States but has a number of highly important cereals now in commercial use that have been bred for disease resistance in such manner as not only to result in practical control of the specific disease but clearly proved to be better yielders and of better quality than the varieties previously in use. From a study of the breeding plots at these three institutions, it must be clear to any intelligent investigator that there is probably no type of cereal plant which cannot be very much improved by breeding and selecting processes now in use; and that by intelligent action one can very greatly hasten the coming into use of types which are far more suited to local conditions than those now commonly sown.—H. L. BOLLEY, for the General Committee, Agricultural College, Fargo, North Dakota.

PHYTOPATHOLOGY

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BACTERIAL STALK ROT OF CORN[1]

H. R. Rosen

In 1919 the writer (14) called attention to a little known disease of corn prevalent in Arkansas. This was followed by two short papers in which brief descriptions of the disease and the pathogene concerned with it were presented. The present paper aims to present a thorough but succinct account of the disease and the pathogene.

DISTRIBUTION AND EXTENT OF DAMAGE

Subsequent to the first communication (14), the disease has been reported by other investigators from the following states: New York, North Carolina, Mississippi, Louisiana, Ohio, Illinois,[2] North Dakota, and Arizona.[3] It is possible that in some instances this disease may have been confused with some other malady, but it is quite probable that the disease is present in other states, as well as in most of those mentioned above. As far as Arkansas is concerned, and probably other southern states, the pathology of corn in general is hardly fathomed as yet.

As to the presence of this disease in other countries, there are no published records known to the writer with the exception of that by Dawson (6) of East Africa, whose report is so brief that there is some question as to the disease described by him being the same as that here discussed.

Evidence will be presented later which clearly indicates that this is a disease which does damage only during moist, muggy, warm weather, and that regions in which the air temperatures are much below 27° C. during the growing season are likely to be free from it. The disease is very probably present in all the states along the Mississippi River from Illinois southward.

[1] Published with the permission of the Dean of the College of Agriculture, and Director of the Arkansas Agricultural Experiment Station.

[2] U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul. Supplement 21. 232–233. 1922. (Mimeographed.)

[3] *Ibid.* Supplement 27. 238–239. 1923. (Mimeographed.)

The writer has noted this disease in Arkansas every year since 1919 (6 years). Field observations have been made and specimens received from a wide area of the State, including the Mississippi River (eastern), as well as the Ozark (western) section. During the years when summer rainfall is quite low, the disease does very little damage and does not cause losses of more than one to two per cent. But when the late June, July, or August precipitation is more than usual, as occurred in some sections of the State in 1920 and in 1925, the disease is by far the most destructive one of corn in the State. I have already recorded losses of from 10 to 30 per cent of the stalks in some fields (15). In 1924 the disease was noted in epidemic form at Fayetteville, Ark., in a small garden of sweet corn—11 out of 20 hills of Golden Bantam having the disease and other varieties being affected to a lesser extent. During 1925 quite a few reports and specimens were received from a number of counties in the eastern part of the State, including Randolph, Independence, and Jackson Counties. Some of the correspondents who reported considerable damage, and who also sent specimens for identification, wrote as follows: "I am sending you specimens of corn stalks that have become affected with what appears to be stalk rot. This corn appeared to be in a healthy condition until a few days ago. Now many stalks are falling over and are being attacked to a more or less extent." (Extract from letter of E. Y. Fitch, June 29, 1925.) "This is the only field I know of that is damaged, but there is considerable damage in it." (Extract from letter of J. E. Critz, June 27, 1925.) "I am sending you herewith specimens of stalks of corn that have us somewhat puzzled as to what is the cause of the rotting off just at the top of the ground. This condition is quite prevalent in this county (Independence County), especially in rich delta or bottom lands—and in some instances is materially affecting the stands. This corn has not suffered seriously from moisture, nor has the temperature exceeded about 98 to 100 degrees F., and at this time there is plenty of moisture, but not excessively wet." (Extract from letter of S. C. Knight, June 27, 1925.)

As to the damage done by this disease in other states, Edgerton's report[4] is of interest. "The bacterial stem rot of corn described by Rosen from Arkansas has been known in Louisiana since 1908. I saw it the first year that I was in Louisiana and have seen it to some extent nearly every year since. Some years it is very troublesome in some localities and other years it is of little importance." This corresponds closely to the writer's observations in Arkansas. From Illinois, Tehon (20, p. 197) reports as follows on the disease—"It was found in Illinois in 1921 in two counties, Jackson and Randolph, where it was reported as serious in the fields affected by it. In

[4] U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul. Supplement 21. 232–233. 1922. (Mimeographed.)

1922 it was again found in small amounts in a single field in Union County, where it was reported to be causing the death of one per cent of the stalks. In 1923 it was seen in Alexander County, where an examination of 100 acres revealed two infected stalks." Roy C. Thomas, in a letter to the writer dated September 29, 1924, reports the disease on Golden Bantam and Country Gentleman in Ohio.

As already indicated, the disease is to be looked for primarily in the southern portion of the corn growing section, although it appears that it may develop to some extent farther north (probably during exceptionally moist, warm periods). In addition to the varieties previously mentioned (15) as susceptible to the disease, the following sweet corn varieties have been found susceptible: Golden Bantam, Stowell's Evergreen, and Country Gentleman.

DESCRIPTION OF THE DISEASE

The writer has already discussed the symptoms in former publications (14, 15). Additional information enables me to make some corrections and

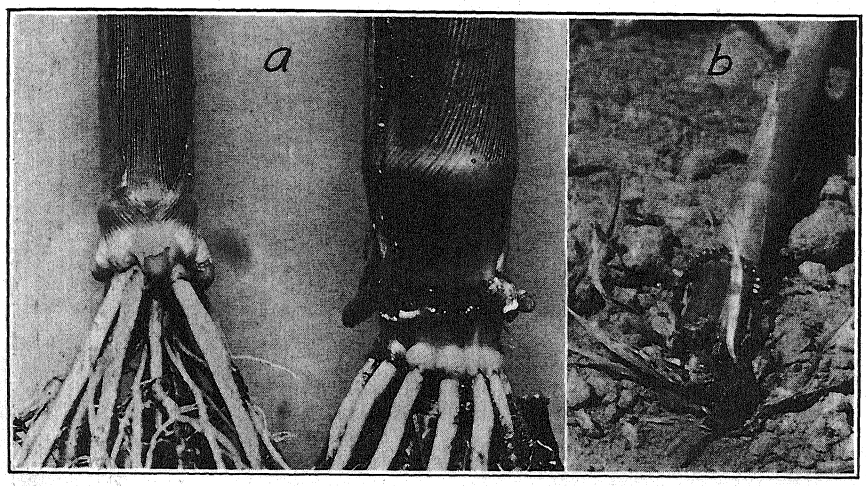

FIG. 1, a—Plant on right artificially infected at a lower node, the resulting rot involving several adventitious roots as well as stalk tissue. Plant on left served as check.
b—Natural infection at a lower node which included root infection.

additions to these. The disease is primarily one affecting the stalks and leaves. The name "root-rot," that was first applied (14), is misleading. It was used because the first observations showed the roots as the main seat of disturbance, but further study has convinced the writer that this was due to some other cause which happened to accompany the bacterial disease in the field where it was first observed. The compact, matted appearance of the roots, as first described, has not been observed in any subsequent ma-

terial, and the writer is inclined to think that this was largely due to wire-worm injury, the matting of the roots representing successive efforts on the part of the host to replace the elongated roots cut short by the wire-worms. Also, too much emphasis was placed on the purplish-brown discoloration of the interior of lower nodes, this feature being more or less common to a number of root troubles (and perhaps non-parasitic root troubles), and is

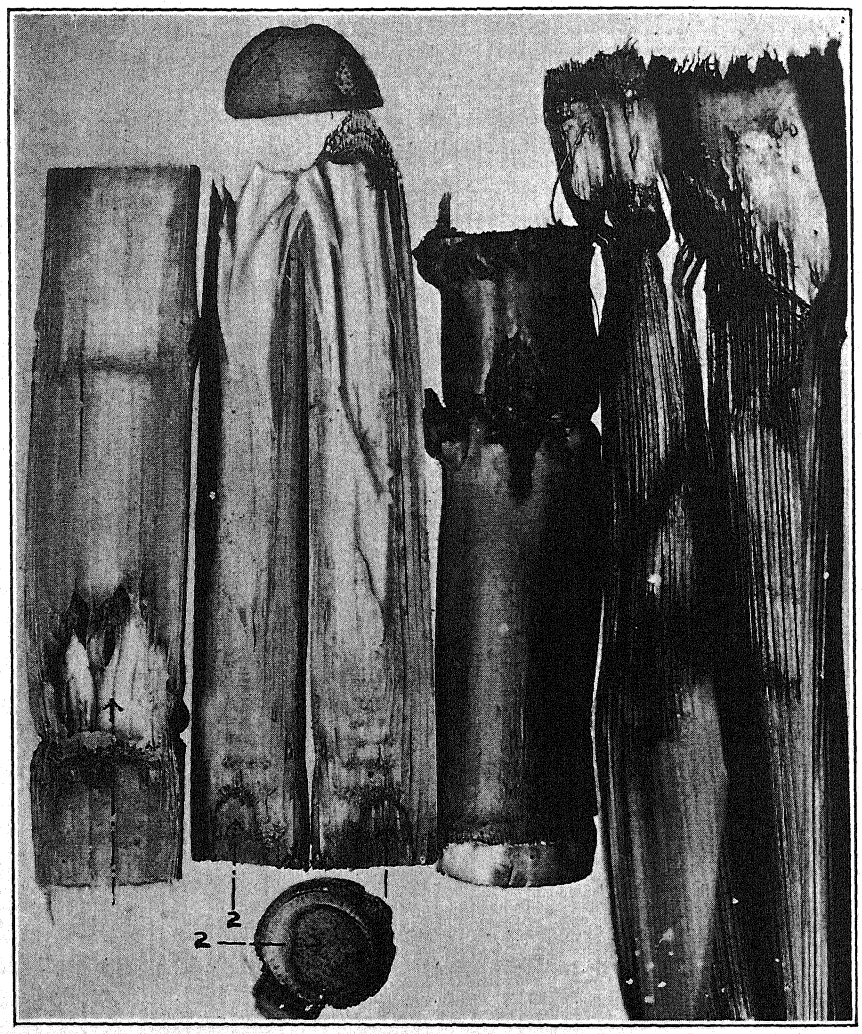

FIG. 2.—Natural infections; arrow at extreme left pointing to internal pocket of dry rot or later stage, other arrows (2) pointing to an early, wet rot stage; sheath at the extreme right showing discoloration and disruption of tissue occurring at an infected node.

only significant in this disease when accompanied by a characteristic rotting of the same or adjoining tissue (Fig. 1).

Briefly stated, the symptoms are as follows: (a) A light-brown or dark-brown rotting, and premature dying of the lowermost leaves (upper leaves rarely become infected), usually beginning in the parts joining the stem and confined primarily to parts of the sheath (Fig. 2); (b) a yellowing and withering of the leaf blades whose sheaths have become rotted; (c) a rotting of the stalk, usually at a lower node (Fig. 2), often at the node immediately above the soil line; the rotted part in severe infections is a dark-brown or blackish, soft, putrid, sunken mass, often with a grayish-white slime in recent infections, and light-brown, shriveled and broken up in older infections (Pl. XVII). In most cases the infection does not extend through the whole thickness of the stalk, but either confines itself to a small localized blackish spot with a water-soaked margin, extending not more than 0.25 inches into the interior and invading only a small part of the nodal circumference (Fig. 4), or the infection, beginning at some external point on the node, extends into the interior, invading a considerable part of this without showing much at the surface; or lastly, the rot may involve the whole circumference of the node and extend but a short way into the interior. As formerly reported (15), the complete rotting of a lower node and the subsequent falling over of the stalk are symptoms of this disease in severe infections (Fig. 3). Roots which happen to be attached to those parts of the stalk which are diseased will likewise become infected, but it should be noted that this disease is primarily a stalk and leaf rot and not a root rot; likewise that leaf blades in contrast to leaf sheaths are rarely infected, though the author has often succeeded in infecting these artificially (Fig. 5).

It is often difficult to distinguish this disease on the leaf sheaths from leaf spotting due to other causes, particularly those spots which are associated with the presence of pollen sacs and aphis honey dew in the ventral side of the sheath, and which, according to Durrell (8 and 9), make it possible for normally saprophytic fungi and bacteria to penetrate the sheath and produce discolored spots. However, such spotting, in the writer's observations, is found mostly on the leaf sheaths, while the bacterial disease here described is conspicuous as a stalk infection. Furthermore, the bacterial disease is found on young as well as on older plants (Fig. 3), while Durrell notes that the Diplodia dry rot fungus is found mostly on older plants.

What is the explanation for the prevalence of this disease on the lower parts of the plant? In other bacterial diseases which are not systemic and which are not particularly associated with insect dissemination, primary infections are also apt to appear on parts close to the soil line. Black rot

of cabbage, black leg of potato, and angular leaf spot of cotton immediately come to mind as good illustrations of this. The same thing is true for a large number of fungous diseases. Closeness or contiguity to soil is the main explanation, the pathogene either being associated with the seed or more directly with the soil. In the latter instance the pathogene lives over on formerly diseased material of past crops or, rather rarely, lives a more or less independent existence in the soil. In these instances dissemination is largely by driving rains or by surface drainage. The angular leaf spot of

FIG. 3.—Typical stalk rot close to the soil involving an extreme disintegration of localized stem tissue.

a—Rot of older plant.

b—Rot of young plants; plants prostrate as a result of extreme rotting. Photographed at Burdette, Ark., June 27, 1921.

cotton is a very good illustration of this. In addition to closeness of soil, the frequency of injuries around the crown of the plant, either in the form of natural wounds or in the form of insect and other injuries, also play an important rôle in rendering the lower parts of the corn plant more subject to disease.

ISOLATION AND ARTIFICIAL INOCULATION EXPERIMENTS

It has previously been reported that the pathogene is rather easily isolated (15). During the six years that have elapsed since its discovery, it has been isolated more than 50 times from natural and from artificial infec-

tions. Owing to the great abundance of bacteria in infected portions, a small bit of material from any portion of the rotted tissue is usually sufficient for microscopic examination. Under the pressure of a cover glass, grayish-white clouds of the bacteria ooze out of such tissue floating in a drop of water. Not infrequently other microorganisms, including various fungi and comparatively large-sized or motile bacteria, are also to be seen in rotted tissue, but if care is taken to choose only freshly infected tissue, these small, plump rods are quite frequently the only microorganisms to be observed. Within the parenchymatous tissue of the corn stalk, which it will be recalled occupies a considerable part of the stalk, the bacterial pathogene thrives and multiplies rapidly under suitable conditions of moisture and temperature, and infected cells are filled with bacteria. The writer has never observed the pathogene in the vascular tissues, and, as the rotted region is always more or less limited in extent, the disease may be considered as a localized one. It may be possible to have localized infections of vascular systems, but the fact that this disease often leaves only the shredded fibers or vascular elements at the rotted joints is external evidence of the lack of vascular infection (Pl. XVII, a). Conceivably, should any injury occur to the xylem of the amphivasal corn bundle so that the phloem would be exposed, it also might become infected.

Isolations by means of platings from internal tissues, either without subjecting this interior tissue to a germicide, or submitting it to the action of mercuric chloride for the customary minute or two and then washing in sterile water, are very likely to result in the development of one type of colony (Pl. XVIII). In order to be certain that any fungus, which might be present and which might be lost by bacteriological dilution culture methods, is isolated, bits of unmacerated tissue were surface sterilized, and plated out directly on various media, especially potato glucose agar and nutrient agar. This, in conjunction with the dilution plate method, should enable one to isolate fungi as well as bacteria which may be present. As a result of a large number of such trials, the writer was able to establish early in his work the presence of a certain bacterium in the diseased tissue. Of the fungi which have been encountered in such rotted areas, *Rhizopus* spp. and *Fusarium* spp. lead in frequency. But inasmuch as the tissue harboring these fungi was always teeming with bacteria, while many isolations revealed no fungi, there is no good reason for considering these other than as secondary invaders. Occasionally other species of bacteria have been isolated from diseased material, but having once learned to distinguish the pathogene, other bacterial colonies which appeared sporadically were readily explainable as secondary invaders or as contaminations.

Artificial inoculations have been made on somewhat more than 2,000 plants, involving about 150 separate trials in the field and greenhouse, and

typical symptoms have been obtained from approximately 20 per cent. It was discovered early in the work that infections were not always obtainable. These failures at times could not be ascribed to any lack of virulence on the part of the organism, as success and failure occasionally occurred in different trials made but a few days apart. As failures became at times more common, and typical infections discouragingly rare, it seemed necessary to make a thorough study of environmental factors as related to disease inception and development. Furthermore, sporadic outbreaks of the disease under field conditions, which were noted at times as regular epidemics in some localities, followed by a more or less rapid and complete subsidence of the disease, made it all the more desirable to undertake such a study.

Dr. Charles F. Hottes, of the University of Illinois, very kindly permitted the writer to use the excellent control apparatus which he had constructed. This consists of a series of large glass chambers in which temperature and humidity, and light to some extent, can be accurately controlled. Time permitted only a study of the effect of temperature on infection. Three different series were run in these chambers at different times, and the results check up closely in the three. As far as humidity is concerned, it had previously been ascertained that little or no infection was secured in a dry atmosphere during the incubation period, and that to insure infection a very moist or practically saturated atmosphere was necessary. This was obtained by wetting large strips of filter paper and placing them on the inside of large bell jars next to the walls. All plants, checks as well as those inoculated with bacteria, were treated the same way. Inoculations were made by spraying the plants with fresh cultures of the bacteria growing upon nutrient agar slants. The spraying apparatus consisted of sterilized, all-metallic artist's syringes. Wounded, as well as unwounded, plants were treated, the wounds consisting of needle pricks and knife incisions on stems, usually at the base.

Summarizing the results of these experiments, it was found that below 20° C. there were no infections; between 20° and 25° C. infections were few and much limited in the size of tissue affected and in the severity of infections; between 25° and 30° C. infections were more numerous, the size of infections greater, and the discolorations and disintegrations of the affected cells much more severe; between 30° and 35° C. the greatest number of infections was obtained, the affected area showing the greatest amount of disintegration and death of tissues.

A portion of the notes of one of these series reads as follows: December 1, 1922, 11:00 A. M., sprayed one pot of corn (Neil's Paymaster) with Burdette strain, another pot with Jackson County (Illinois) strain, and one with sterile water. Three such pots placed in each chamber at the following temperatures: 15°, 20°, 25°, 30°, and 35° C. Bell jars lined internally with wet filter paper placed over each pot.

December 2, 9:00 A. M.—35° chamber reads 36° C. Others O. K.

December 3, 8:30 A. M.—35° chamber reads 40° C. Others O. K. Bell jars removed. In 30° chamber, yellow tips noted on several leaf blades of pots of both strains, one leaf showing withered area around margin.

December 4, 8:30 A. M.—Plants in 35° chamber showing brownish-yellow tips in pots of both strains, no leaf discoloration of plants sprayed with sterile water. Same observations on the plants in the 30° chamber. In the 25° chamber, two leaves with yellow tips in the pot sprayed with Burdette strain, none with Jackson County strain. No discolorations at 20° or at 15° C. At 25° one tip of a true leaf and one coleoptile tip of another plant infected, infection being in the form of yellowish-brown discoloration extending 1 cm. downward and involving the whole width of the leaf and coleoptile, with an indefinite lighter colored border. Microscopic examination shows brownish tissue teeming with bacteria, which ooze out in thread-like whorls in a water mount.

At 30° C., pot sprayed with Jackson County strain showing one leaf with dark brown withered tip and with light-yellow border extending about one-half way down the leaf and farther, extending as a broad, yellow streak along the midrib. Pot sprayed with Burdette strain showing five leaves of as many plants infected. Symptoms comparable to those given above. Each leaf carefully examined under the microscope and found to harbor bacteria in the discolored and dead areas.

At 35° C., pot sprayed with Jackson County strain shows four true leaves on as many plants badly diseased at the tips and extending along the sides for several centimeters or involving most of the leaf area. Same symptoms as at 30° C. Each infected leaf examined under the microscope and bacteria found in each. All coleoptiles brown, and more or less disintegrated near the soil line. Pot sprayed with Burdette strain shows six leaves badly infected at the tips and sides. Four others with infections just beginning at the tips. No fungi were noted in the whole of the above recorded plants and the bacteria appeared as the typical short, plump rods. All check plants sprayed with water remained healthy.

December 5, 8:30 A. M.—Temperature of 35° chamber reads 33° C. Others O. K. No infections noted other than those recorded above.

December 6, 8:30 A. M.—Pot kept at 35° C. and sprayed with Burdette strain shows one seedling with dead stem, the brown water-soaked tissue extending from below the soil line to above the node, 1.5 to 2 cm. in length. Microscopic examination shows tissues teeming with bacteria; no other microorganisms found. All other plants showing no stem rot.

December 7, 8:30 A. M.—Pot at 30° C. sprayed with Burdette strain shows three seedlings with brown discoloration at the base of the stem, the discoloration extending into the interior. Microscopic examination shows plenty of bacteria in the tissues; no other microorganisms observed. Pot at 30° C. sprayed with Jackson County strain shows one plant, previously wounded at the base with a knife, with brown discoloration and rotted tissue at base of stem. Microscopic examination reveals bacteria present. Wounded, uninoculated plants appear perfectly sound, with no noticeable discoloration.

These studies clearly show that temperature plays a very important rôle in the inception and development of the disease. Attention is called to the fact that both air and soil temperatures were under control in these experiments. They fully confirmed field observations and the numerous other trials at artificial infection made in the field and in the greenhouse, in which it was found that no infections occurred unless the temperature was fairly high.

This naturally leads to the question as to direct effect of temperature on the growth and health of disease-free corn and on the pathogene itself. A number of investigators have studied the effect of temperature on corn, particularly on seed germination and on seedling development. The careful work of Lehenbauer (10) and of Dickson (7) is especially worthy of note. The former made an intensive study of the effect of temperature on the growth of corn seedlings, using Dr. Hottes' control apparatus. He finds that the optimum temperature for the growth of shoots of maize seedlings in water culture for a 12-hour period is 32° C. (89.6° F.). At high tem-

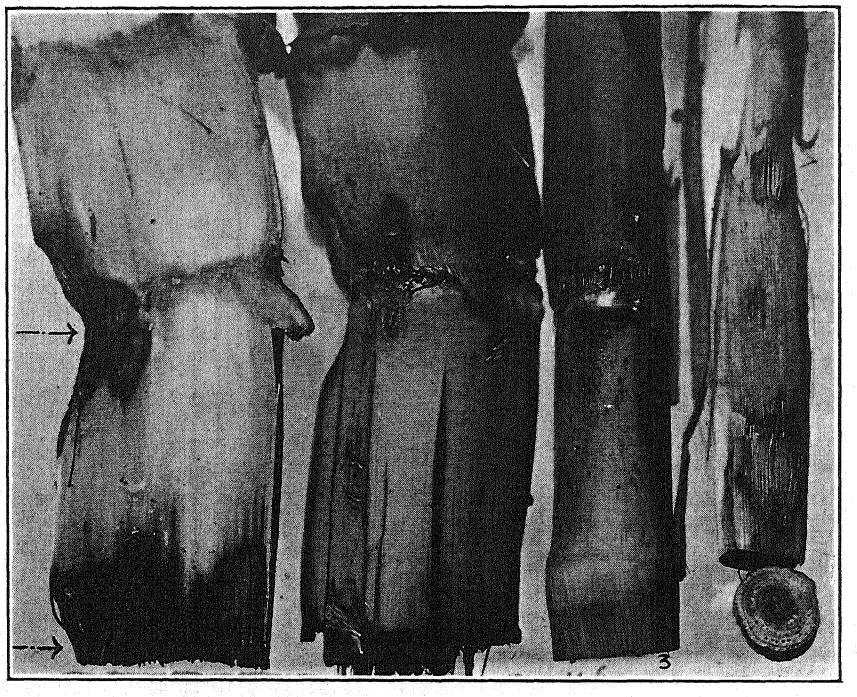

FIG. 4.—Arrows at extreme left point to rotted areas, internal view, followed by external view (to the right). No. 3 represents a nodal lesion which has not progressed to any extent into the interior. Extreme right hand figure shows spots on sheath.

peratures, 31° C. and above, the growth of these shoots is not maintained, there being a marked falling off in the rate during prolonged periods of exposure. He also finds that there is no such thing as an optimum temperature for corn seedlings, but rather a time-temperature relationship, and states that "the optimum temperature for growth under these conditions is found to change as the length of the period of exposure is altered." The growth rate at medium temperatures accords with the van't Hoff law, show-

ing a doubling of the rate for each rise of 9° or 10° C. Dickson finds that corn seed germinates more rapidly at 32° C., seedlings develop most vigorously and most uniformly at the soil temperatures of 24° to 28° C. Corn matures faster at comparatively high temperatures, the floral parts developing first at about 28° to 32° C., while at soil temperatures below 16° C. he was not able to bring corn to maturity. In general, he finds that corn functions best at rather high soil temperatures. While there does not appear to be a close agreement between the results of Lehenbauer and those obtained by Dickson, this is, at least in part, to be explained by the differences in treatments and in standards of measurement, one using both soil and air temperatures and the other using primarily soil temperatures. For the present, in the absence of further work, it may be assumed that, other factors being favorable for good growth of corn, comparatively high soil and air temperatures are best suited to its vegetative vigor and, perhaps, productiveness.

In order to determine the effect of temperature on the growth of the pathogene, several nutrient agar slants were inoculated with fresh cultures of the two different strains and placed, in duplicate, in the 20°, 25°, 30°, and 35° chambers used at the same time for one of the artificial infection series. At the end of 18 hours there was good growth at 35° and at 30°, noticeably less at 25°, and very markedly less at 20° C. As will be shown more fully later on, the organism grows very rapidly under suitable conditions.

It appears then that temperatures that are most favorable for the growth of corn are also most favorable for the growth and infectivity of the pathogene. Field evidence supports these greenhouse and temperature chamber studies. In all the more or less serious outbreaks of the disease it has been found that the weather was very warm and moist during the attacks; and conversely, in the absence of such weather, very little of the disease has been noted. Associated with this, the lower latitudes found in the eastern part of the state, particularly along the Mississippi River, in contrast to the western or hilly section, showed more frequent outbreaks and the disease was present in its worst forms, *i.e.*, complete rotting of a localized portion and a subsequent falling over of the stalk.

While temperature undoubtedly plays a very important rôle in the development of this disease, humidity, as already indicated, apparently plays just as important a rôle. While no experiments have been conducted with adequate controls to substantiate this statement, all the observations on artificial and natural infections tend to show it to be true. United States government records of precipitation and humidity of Mississippi County, Arkansas, for August, 1920, are of interest. (This was the locality and period in which 10 to 30 per cent of the corn was found ruined.) Only 6

clear days are reported for the whole month, while 5 days are listed as partly cloudy, 20 days cloudy, and 5 days with precipitation. Although the total amount of rainfall was only 4.5 inches, being 1.54 inches more than normal, the large number of cloudy days is perhaps of greater significance. Numerous comparable records could be cited which clearly show that a high humidity is indispensable. For example, the very best artificial infections, *i.e.*, those closely resembling natural stalk infections of the severe form, have been obtained in the field only when rainy weather set in immediately after the plants were inoculated. This was particularly noticeable during the last days of June and the first few days of July, 1925 (Fig. 3). Under greenhouse conditions, on the other hand, in which corn does not make as

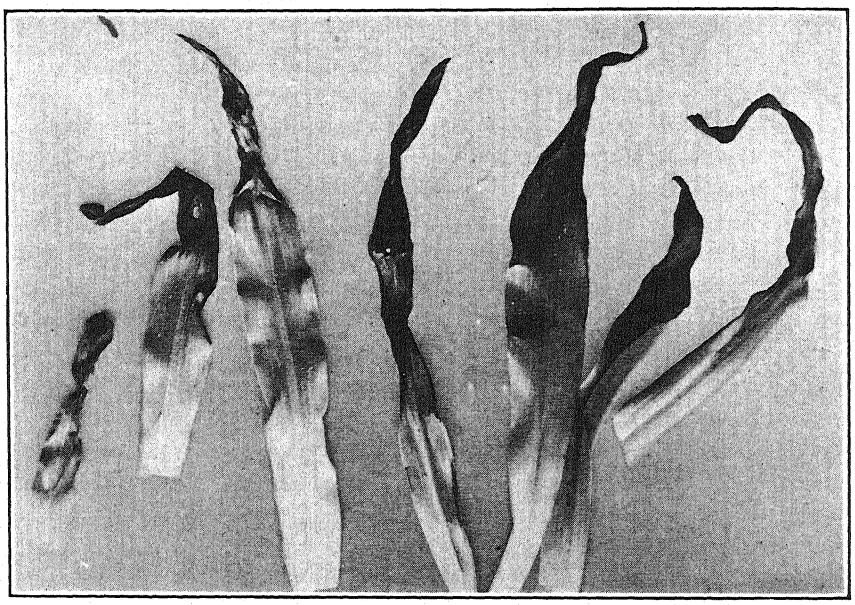

FIG. 5.—Artificial infections on leaf blades in form of dark- or light-brown, withered tips, inoculum in the form of a young culture suspended in water, applied as a fine spray by means of a sterilized, all-metallic artist syringe. Check plants sprayed with sterile water, otherwise treated in same way as inoculated plants, had healthy leaves.

vigorous growth as under proper field conditions, the extreme forms of stalk rot usually have not been observed, although infections were to be had. The explanation for this appears to be something more than the necessity of moisture for bacterial invasion, since plants covered with bell jars for 48 hours or more have been kept with moist leaf surfaces and with little or no extreme stalk rot developing. Occasionally severe infection has been obtained [(14), Fig. 3]. It appears likely that conditions which favor suc-

culence of the host, in which a comparatively large amount of soft, parenchymatous tissue and a small amount of hard woody tissue is being formed, are the direct factors which predispose a plant to infection and extreme necrotic developments. In this respect the well-known fire blight of pears may be cited as a disease comparable to the bacterial stalk rot of corn.

PATHS OF INVASION

In artificial inoculation experiments with seedlings, infections often appear on the leaf blades in which the inoculum is applied as a spray. This is to be expected, as very little actual stalk surface is exposed. Very often these infections start at the apex, indicating entrance of the pathogene through the apical hydathode (Fig. 5). Microscopic examination of such infections invariably reveals the pathogene in great numbers within the infected tissue. In addition to this form of invasion, isolated spots and streaks of blades and sheaths indicate other paths, *i.e.*, stomata or wounds. Invasion by means of leaf stomata is apparently rare, although it occurs at times. In contrast to this, when environmental conditions were favorable, the author has obtained excellent infections through stomata situated in the lower portion of the stalk (Pl. XIX, b). In this region the stomata are much more common than may be supposed. Careful studies of natural infections seem to indicate that this is one of the most common ways in which the bacteria gain entrance into the stalk. In order to test this out, a number of stalks growing in the field were inoculated by means of needle smears around the crowns, care being taken not to wound the plants; these smears were then covered with moistened absorbent cotton. Other stalks were treated in like manner, with the exception that needle pricks were made and the organism smeared in and around these. The number of infections obtained by smears was fully as great as that obtained by pricks. In these experiments, also, it is worth noting that in periods of dry weather infections by either method were few.

As far as wounds are concerned, the writer has previously (15) called attention to the natural wounds common to the corn stalk at the lower nodes, when endogenous roots are pushed out. Artificial infections have been obtained in these regions (Fig. 1), and examinations of natural infections have shown a number of cases where infections appeared to have started in tissue ruptured by the passage of one or more roots. Early infections of this sort appear as light-brown water-soaked areas of the leaf sheath and of the adjoining stalk tissue surrounding the root. Injuries caused by various insects also have been found to offer excellent paths for infection.

A histological study of diseased tissue reveals discolored cells which, depending upon the age and progress of infection, show various stages of disintegration. In young infections there is little disintegration, the bac-

teria being found within the cells and in the surrounding intercellular spaces. In older infections, however, many of the parenchymatous cells are more or less completely broken up and disjointed, and only remnants of tissue may be observed with cells appearing more or less intact. The extreme rotting and loss of cell cohesion makes the disease comparable to the soft rot of vegetables due to *Bacillus carotovorus* Jones or to the Rhizopus rot of various fruits and vegetables; a study of the enzyme action of this pathogene in all probability would reveal enzymes that are capable of acting upon pectic compounds.

THE PATHOGENE

Morphology. The organism is a short, plump, rapidly growing rod (Pl. XIX, c). (As the organism is a marked acid and gas producer and has other characteristics of *Bacillus coli,* it seemed desirable to compare these in various ways, and the results of such comparisons will be presented.) At temperatures of 25–30° C. growth occurs within 12 hours on suitable media, while in 24 hours profuse, heaped up, colonies usually appear on agar slants or on plates. With the exception of one glaring error, the brief description previously given (16) for this pathogene characterizes it quite well. The statement that it is motile by means of a single polar flagellum is incorrect and was based on stains made according to the Van Ermengem flagella staining method (Pl. XIX, a). By this and other methods, such as the Pitfield's, long streamers are often obtained, drawn out from one end of the organism. Subsequent studies enable me to say that these threads represent a slimy matrix or sheath surrounding the organism, which, when spread out on a cover glass, may be observed as masses of intertwining, coalescing threads and ramuli, which surround one or more organisms, frequently simulating flagella-like processes. As most if not all of the well-known flagella staining methods engender deposits of greater or less amounts of precipitate on and around the microorganisms, often making it impossible to obtain a clear view of them, it is not very surprising that this slime should have been interpreted as flagella. It will be shown elsewhere by the writer that at least in one other bacterium, supposedly one of the best known, the flagella have been incorrectly described. The fact that this organism in pure culture never appears motile, when observed in a hanging drop, constitutes good supplementary evidence. On the other hand, one of the reasons for the earlier belief of motility was due to the presence of actively motile bacteria, along with non-motile forms, in some natural infections. These are now regarded as secondary invaders. However, studies of flagella on a fairly large number of diverse bacteria, in which considerable variation has been observed in number and arrangement of flagella in the same species, and also the variations obtained in physiological processes

of different strains of the same species, are sufficient to make the author hesitate about any generalizations concerning motility in all possible strains. For the present, the writer can report that, in all strains of the stalk-rotting organism studied thus far (about 25 in number, but representing only two states, Arkansas and Illinois), no motility has been observed in pure cultures.

As previously recorded, the average measurements of the bacteria stained by ordinary methods are $0.7-1.2\mu \times 0.5-0.9\mu$. The organism is frequently found in groups or clumps—doubtless due to the slimy envelopes—often in pairs, and rarely in chains. Capsules are present (Pl. XIX, d) as may readily be observed by using the Plimmer and Paine method (13). By this method, including the enveloping capsules, the average measurements are $1.5-1.8\mu \times 1.0-1.3\mu$. No spores have ever been observed. Many young and old cultures have been variously stained, with no indications of spores. As the pathogene takes the form of rather small sized rods, spores would perhaps not be expected. It is Gram negative, using the method described by Burke (4). *Staphylococcus aureus* with the same stain gives a positive test. Counter-stained with safranin, the corn organism stains red, while *Staphylococcus* remains deep blue. It is not acid fast. A broth culture, 24 hours old, stained with Ziehl's carbol-fuchsin and then treated with 20 per cent sulphuric acid for a moment, shows no color. When counter-stained with aqueous methylene blue, the organism appears blue with no trace of red.

CULTURAL CHARACTERS

Agar plates. On peptone-beef agar poured plates kept at 25° C., many of the colonies become visible in 24 hours, and in 48 hours most, if not all, are visible. They are white, round, smooth, glistening, opaque, have a consistency of melted butter or of boiled starch; the elevation is convex, edge entire, and the structure amorphous. Deeply imbedded colonies, next to the glass, are flat, spreading, and have a tendency to form concentric rings. When held up to a bright light, the colonies appear brilliantly fluorescent. After three weeks, when the medium is well dried out, colonies show a marked tendency to form a flat, spreading growth, zonated near the margin, the margin noticeably lobed and scalloped.

Agar stroke.—At 25° C. there is a good growth in 24 hours, reaching a width of 4 to 5 mm. at the base of the slant, and tapering upwards. The growth is filiform to spreading (Pl. XVIII, b), raised or convex, glistening, smooth, butyrous, and opaque. (One strain isolated from Burdette, Arkansas, showed a brownish color developed in most of the common media.) Odor is marked in some strains and less evident in others, resembling putrid decaying matter.

Agar stab. Growth occurs along the entire length of the stab, somewhat better above; gas bubbles produced along the line of puncture (Pl. XVIII, c) and at times extending out through entire thickness of the medium. As a result the medium becomes cracked. Growth finally occurs in the pockets made by the gas bubbles. By suspending pieces of filter papers soaked with Nessler's reagent over cultures and heating, a brownish color appears on the paper, indicating that these gas bubbles were probably ammonia.

Gelatin plates. Growth at 17–20° C. is rather slow and not copious; colonies are round, white, with entire margins. There is no liquefaction.

Gelatin stabs. At 15–20° C. growth is observable down the whole length of the stab at the end of three days. There is no liquefaction at the end of five weeks. The line of growth is filiform, and there is a tendency for gas formation (ammonia) observable as bubbles near the line of growth.

Nutrient broth. In peptone-beef broth testing pH 7, kept at 25° C., there is a noticeable clouding and a slight precipitate, but no pellicle at the end of 24 hours. In 48 hours there is a great surface growth mainly observable in the form of a ring and floccules, or compact slimy masses and streamers. Fine whitish granules are formed in most strains (absent in some); clouding gives place to a marked turbidity with numerous floccules; the sediment is abundant and rather cohesive in most strains. In many strains there is a marked putrid odor. In contrast to the corn organism, *Bacillus coli* forms a much heavier surface growth; the sediment is never viscid (at least in the strain here used) and breaks up into compact flaky particles.

Nutrient broth over chloroform. As compared with growth on the same medium in free air, there is no clouding in 24 hours, although the precipitate is about the same. In 48 hours there is no surface growth, and the granules are not nearly as abundant as in free air; the sediment is just as heavy but more compact. At the end of six days, growth still appears considerably restricted.

Nutrient broth plus alcohol. Absolute alcohol was added to nutrient broth, so as to make 2, 3, 4, 5, 6, 7, 8, 9, and 10 per cent solutions. Growth occurs in 2, 3, 4, and 5 per cent solutions, with a heavy surface pellicle, something which does not occur in ordinary broth. At the end of three weeks, when considerable alcohol has evaporated, surface growth occurs in all up to 10 per cent, indicating that, while the alcohol at higher percentages may have considerably retarded growth and perhaps killed some of the organisms, it did not kill all the organisms at the concentrations used.

Sodium chloride in nutrient broth. At the end of three days, growth appears to be equally good in concentrations up to 3 per cent. At 4 and 5 per cent there is very little growth.

Loeffler's blood serum. Growth is restricted and whitish. There is no liquefaction.

Lima bean agar slants. Growth in 24 hours is very heavy, superior to that on nutrient agar in the same time interval, the greater growth being even more noticeable at the end of 48 hours. On this medium, growth is more spreading, less opaque, and there is a greater tendency for gas production. The medium was made up according to the directions given by the writer in a previous publication (17, p. 369).

Uschinsky's solution. Growth is good; fluid is not rendered viscid.

Fermi's solution. No growth at the end of five days.

Potato cylinders. At 25° C. there is good growth in 24 hours. It is quite heavy, heaped up, creamy-white, glistening, and not slimy. Growth is best on the moist part of the medium at the base of the cylinder. *B. coli* shows a similar type of growth but is somewhat darker in color, grayish white and flatter, with a greater tendency to spread. After the corn organism had grown on the cylinders for about a month, the cylinders were macerated, 10 cc. of distilled water added, and 1 cc. of an alcoholic solution of iodine. A purplish red (very near dusky auricula purple of Ridgway) immediately developed, indicating a partial starch hydrolysis. Cylinders upon which *B. coli* had been grown for the same length of time, and which were treated the same way, developed a deep blue color, comparable to the color of uninoculated and similarly treated cylinders.

Nutrient broth plus 1 per cent potato starch. Growth is very heavy at the surface in the form of an adhering pellicle. At the end of 11 days it was tested for starch hydrolysis by the same method given for potato cylinders. A purplish blue color appeared immediately, which gradually changed to a reddish blue, finally losing all color by standing over night. Similar treatment of growths of *B. coli* remained deep blue. This test and the one on potato cylinders indicate that the corn organism possesses diastatic action.

Potato dextrose agar slants. At 25° C. growth is copious, raised, shining, and white. In some strains there is a marked viscidity, the growth pulling away from the medium in thread-like fashion when touched with a loop. No such sliminess was noted in the strain of *B. coli* studied. In 48 hours there is a noticeable production of gas in the corn organism and much more in that of *B. coli.*

Milk. At the end of three days at 25° C. milk begins to clear in the form of a watery, honey-yellow band appearing at the surface. Coagulation is delayed, but becomes quite marked by the sixth day; color of medium is not changed. At the end of the same period cultures of *B. coli* extrude whey, which remains white and does not clear. On the 18th day, upon the addition of methyl red, cultures of the corn organism show but very faint pink, while *B. coli* cultures show a pink of around pH 5.2.

Litmus milk. There is no change in color up to the third day, aside from the watery, honey-yellow surface band, but by the sixth day the lavender color has changed to a pinkish-cream, indicating acid production. Testing with brom cresol purple, with colorimetric comparisons, the active acidity is about pH 5.4.

Indol production. On the 10th day, using the Ehrlich test, nutrient broth gave a positive test for indol. Tests by the sodium nitrate-sulphuric acid method were negative. It is of interest to note that in broth containing sodium chloride there is a decreasing amount of pink in the Ehrlich test as the percentage of salt is increased, being very markedly pink in 1 per cent and only faintly so in 5 per cent. This would seem to indicate that moderately strong solutions of table salt, and probably other inorganic salts, either hinder the production of indol or prevent its detection.

Development of crystals. In old cultures in nutrient broth containing 1 per cent dextrose, there is a noticeable development of crystals. These appear similar to those figured by Smith (19, Fig. 57, No. 2). Tests for tyrosin with Mellon's reagent were negative; likewise tests for tryptophane with bromine vapor gave negative results.

Nitrite production. Growth is fairly abundant within 48 hours in nitrate broth. Some strains develop a compact, viscid sediment. Surface growth is only noticeable as a faint ring. Tests for nitrites with starch water-potassium iodide on the 10th day are positive. Checks gave no tests.

Hydrogen-ion concentration in relation to growth. Nutrient broth was rendered acid and alkaline by adding hydrochloric acid and sodium hydroxide aseptically after the broth had been sterilized. The following series (4 tubes of each) was used: pH 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 7.5, 8.0, 8.5, and 9.0. Growth occurred in the series, beginning with pH 5.0. Below that, at the greater hydrogen-ion concentration, there was no growth. It appears that the organism has a rather wide range with reference to hydrogen-ion concentration and is especially tolerant of alkaline conditions.

Toleration of acids. The following acids were added aseptically to nutrient broth after it had been sterilized: acetic, citric, hydrochloric, lactic, oxalic, and phosphoric. The concentration used for each was 0.1 per cent. The resulting hydrogen-ion concentration was: acetic acid pH 4.9, citric pH 5.0, hydrochloric pH 4.8, lactic pH 4.7, oxalic pH 4.7, phosphoric pH 4.5. Growth occurred only in the citric acid broth, testing pH 5.0. It would take much more work to show whether this means that the corn organism is intolerant of anions other than citrate, or whether the lesser concentration of hydrogen-ions in the citric acid broth was responsible for the growth.

Hydrogen sulphide production. Filter paper saturated with lead acetate hung over cultures in nutrient broth, and in nutrient broth plus 1

per cent potato starch failed to show any color, indicating no hydrogen sulphide production. However, the writer is not satisfied with this test. The recent one recommended by the committee on pure culture studies, Society of American Bacteriologists (5), has not been tried.

Ammonia production. Cultures several days old, in peptonized beef broth testing pH 7, respond readily to a test for ammonia (filter paper saturated with Nessler's reagent). The odor of ammonia is not detected however, possibly because of the very marked putrid odor produced by some strains of the organism.

Gas production. In the presence of carbohydrates the organism is a marked gas producer. This organism also produces gas in the absence of carbohydrates, but this gas, ammonia, is the product of protein decomposition. Gas production in fermentation tubes has been carefully studied, and the results are presented in table 1.

TABLE 1.—*The production of gas by Phytomonas dissolvens growing in fermentation tubes in the presence of carbohydrates*

Carbohydrate	Relative amount of gas in closed arm after 24 hrs.	Amount of gas in per cent after 5 days	Proportion of H to CO_2
Dextrose	Abundant	60 (25)[a]	1:1, (2:1)[a]
Galactose	Noticeable	15	1:1
Mannite	Noticeable	60	1:1, (2:1)[a]
Sucrose	Noticeable	60 (50)[a]	1:1
Maltose	Abundant	50 (30)[a]	1:1, (2:1)[a]
Lactose	Noticeable	25	1:1
Raffinose	Noticeable	25 (25)[a]	1:1
Inulin	Absent	Little in 5 days; 25 per cent in 10 days	
Glycerin	Noticeable	20 (30)[a]	

[a] Figures in parenthesis refer to *B. coli* cultures which were run at the same time.

Acid production. Lactose agar stabs containing Andrade-Penny's indicator kept at 25° C., develop a pinkish color in 18 hours, with considerable gas production in the form of bubbles. The pink color denoting acid development remains after two months of growth. In order to ascertain the active acidity developed by the corn organism in the presence of different carbohydrates, 1 per cent of each of the carbohydrates listed in table 2 was added to the nutrient broth. The acidity was determined by colorimetric comparisons, using the Clark and Lubs indicators.

From table 2 it will be seen that considerable acid is produced in two disaccharides and in one polysaccharide; otherwise the acid production is

not very great. Unfortunately, in the series just presented, no comparisons were made with *B. coli.* However, in a series of agar slants containing litmus as an indicator, and in the presence of various carbohydrates, the original blue was changed to red in six hours by *B. coli,* while the corn organism cultures remained blue, or showed only a mere trace of red or pink. These consisted of the following: galactose, mannite, sucrose, lactose, raffinose, and glycerin. In 24 hours, however, all the carbohydrate media were red—the corn organism cultures as well as *B. coli* cultures. It thus appears that *B. coli* develops a greater acidity in a shorter interval of time than the corn organism.

TABLE 2.—*The acid production of Phytomonas dissolvens in the presence of carbohydrates*

Carbohydrate	Original pH	pH at end of 11 days
Dextrose	6.8	6.0
Galactose	6.8	6.0
Mannite	6.8	6.0
Sucrose	6.8	5.4
Maltose	6.8	5.4
Lactose	6.8	6.1
Raffinose	7.1	6.1
Potato starch	6.8	5.4
Check (nutrient broth)	7.0	7.0

Germicidal action of mercuric chloride. Comparatively equal amounts of the organism, growing in bouillon, were transferred to tubes, each containing 10 cc. of mercuric chloride of the following dilutions: 1:1000; 1:10,000; 1:50,000; 1:100,000; and 1:500,000. At the end of five minutes a drop of each dilution was transferred to a tube of nutrient broth. No growth occurred in the following: 1:1000; 1:10,000; 1:50,000; while the transfers from 1:100,000 and 1:500,000 grew in 24 hours. After 15 minutes exposure, transfers from 1:100,000 failed to grow, while those from 1:500,000 appeared unaffected. It appears, then, that mercuric chloride is a very efficient germicide for this organism.

PHYSIOLOGICAL REACTIONS

Effect of temperature on growth. In connection with the artificial inoculation experiments, attention has already been called to the fact that this organism grows best at around 30° to 35° C. In addition to the study there reported, the organism grown on nutrient broth was subjected to various temperatures below 20° C., resulting in very poor growth or no growth at all. At 15° C. there was a slight clouding in five days; at 10° C.

there is a very faint cloud at the end of 8 days; while at 0° C. there appears to be no growth. Due to lack of facilities, the upper limits of growth have not been determined. Based on records of other bacterial pathogenes, this would probably be about 40° C., with the death point several degrees above that. In reality, such studies as these here reported, and in common use, are of little significance as to the effect of temperature under natural conditions, because, under these conditions, constant temperatures almost never exist. It would be far better to subject the organism to field temperatures at different times of the year.

Effect of drying. Diseased material collected during July of 1921 was kept in a dry condition in the laboratory until late summer and then placed near an open recess in a building where it was sheltered from rain but more or less subject to outdoor temperatures. Here it was left until the first week in July, 1922. A portion was then thoroughly washed, macerated in sterile water, and the suspension sprayed on one pot of corn seedlings. Typical infections resulted particularly in three seedlings that had been wounded. (Wounded plants which were not inoculated remained healthy.) The infected portion of one of the plants was then washed, surface sterilized, macerated in sterile water and plated out. Notes read that in the series of 10 plates, pure cultures were obtained without a single evident contamination. Transfers from the pure culture isolations were in turn inoculated on another group of plants and resulted in two typical infections on as many plants. From one of these the pathogene was reisolated. It thus appears that the organism may readily live over for a year at least in dry tissue.

Overwintering. The above data also indicate one method by which the pathogene may live over winter. As the primary seat of stalk infection is close to the crown of the plant, the diseased part has an excellent opportunity of remaining undisturbed during harvesting of the corn, whether or not the stalks are cut. The stubble remaining on the field serves as a source of infection for next year's corn if no rotation is practiced. Experimental evidence for other means of overwintering unfortunately is not at hand, due to the destruction of inoculated ears on three different occasions. Field data gathered at various places seem to indicate that the pathogene may also be carried in, or on, seed. For example, one field was cleared of timber and was used for growing corn for two years, then alfalfa for seven years, followed by another crop of corn. Upon this last crop the disease was discovered. Of course such evidence is not very satisfactory for various reasons. For one thing, ears showing natural infections have not yet been found. Although ears showing rotted husks and kernels have been frequently observed, some of which harbored bacteria in the diseased parts, all attempts at isolation have yielded either fungi or

bacteria which have no resemblance to the species here discussed. Hence the actual means of disease introduction in different localities remains to be investigated. As artificial infections sometimes are secured on leaf blades and sheaths, and as natural infections on lower sheaths are common, there is very good reason to suspect that natural infections take place on husks at least and also, perhaps, on the kernels themselves. Indeed, in one case a Golden Bantam seedling growing in sterilized soil was infected with bacterial stalk rot. The seed came from the same lot as those which produced 11 diseased hills out of 20, previously mentioned.

Effect of low temperatures and freezing. Three different experiments were undertaken to determine the effect of very low temperatures. First, freshly made strokes on nutrient agar, as well as full grown strokes, were put into a calcium chloride water mixture, operated in conjunction with an ice machine, with a temperature of —17° C. Tubes were left in this for 10, 30, 60, 90, and 120 minutes, and then placed in a 30° C. incubator. After 24 hours of incubation, growth occurred on all the freshly made strokes, while transfers made from the full grown ones also made successful growth. Second, old strokes of two different strains growing on nutrient agar were placed in a melting ice mixture, kept there for two hours, and then placed in a 30° C. incubator. All transfers made from these grew. Third, a loopful of a 48 hour broth culture was placed in each of four tubes containing the same quantity of nutrient broth. Three of the tubes were placed in a freezing brine ice mixture, in which the broth solidified, and was kept in a frozen solid state for two hours. After thawing, poured plates were made from similar quantities of each of the three tubes, as well as from the check. Counts of the number of colonies developed from check as well as from the frozen cultures showed a reduction of about 90 per cent of the colonies in the frozen cultures. Considering the differences in media and in type of treatment, these three experiments are not as conflicting as they appear. While the mortality of the organism in a liquid medium subject to extreme freezing is high, it can, as a whole, withstand very low temperatures.

Effect of sunlight. Poured plates placed on a block of ice in bright sunlight (mid-day of the 22d of June), with one-half of each plate covered with black paper, were exposed for varying periods of time. Those exposed for 30 and 45 minutes showed no difference in number of colonies between exposed and unexposed halves. But those subject to one hour exposure and over were almost entirely killed. Compared to other bacterial phytopathogenes, a number of which are said to succumb after exposure of 15 to 30 minutes, the corn organism is not so sensitive to sunlight.

Vitality on culture media. On ordinary media such as nutrient broth, nutrient agar, and on milk, the organism lives for quite a few months. For

example, nutrient agar stroke cultures which were kept in an icebox for 10 months, during which time the medium dried out considerably, although not losing all moisture, showed growth in 24 hours when transferred to nutrient agar.

Group number. Using the most recent chart (1925) issued by the Society of American Bacteriologists (5), the index number is 5000–32021–1111.

RELATIONSHIP OF THIS DISEASE TO BURRILL'S BACTERIAL DISEASE OF CORN

In the first publication (14) of this disease it was stated that it "has been known since 1889" and that it was first described by Burrill. This statement was based upon Dr. Burrill's description of the disease (2 and 3) and not upon any specimens seen by the writer. Having now made a very complete study of all the specimens left by Burrill at the University of Illinois, the present author can now report that it is very doubtful if the disease or diseases Burrill dealt with is the one here considered. Most, if not all, the material examined showed various blade and sheath spots (Pl. XVII, e), quite comparable to the spots described by Durrell (8 and 9). Only one cornstalk specimen was found, representing a seedling, and in this the symptoms are markedly different from the bacterial stalk rot. Microscopic examination of a number of these spots show various fungi and bacteria present within the tissues, confirming the gross symptoms as to type of disease. All the collections examined, including those having the following numbers, show the ordinary sheath spots: 9153, 10527, 25896, and 32392.

Aside from the question as to whether the disease described by Burrill is the one here discussed, Burrill described, but apparently did not name, the causal organism, although various well-known authors, including Russell (18, p. 36), Migula (12, p. 315), Ludwig (11, p. 95), Voglino (21, p. 37), and quite a few of the more recent authors of text-books attribute both *Bacillus zeae* and *Bacillus secales,* the latter supposedly a synonym, to Burrill. Most of these writers cite Burrill's publications on this subject, and when these are carefully studied it appears very doubtful if the authors ever read Burrill's work. He has only two articles on the corn disease that are of importance (2 and 3) and several others that are merely abstracts and reviews. In none of these does he give any name. The earliest use of the name *Bacillus zeae* Burrill that the present author has been able to find appears in Russell's work (18) in 1892, and it would not be surprising if that author, who was working on practically every bacterial plant pathogene then known and on other types of bacteria, for convenience adopted a name, and used for authority, the name of the man from whom he had obtained his cultures. Certainly, as late as 1891 Burrill had not

given a name to the organism, for on package No. 10527 of Burrill's material is found the following data: "A bacterial disease of corn, described by Burrill but not named. On Zea mays, Urbana, Ill., June 17,1891. C. P. C(linton) Det. McCluer, Coll." This date is so close to the date of Russell's publication, 1892, that the explanation offered above for the origin of the name, or rather two names, seems plausible.

The difference between the disease here discussed and Stewart's disease has previously been presented (14).

TECHNICAL DESCRIPTION

Phytomonas dissolvens (comb. nov.)[5]
(Pseudomonas dissolvens Rosen.)

Non-motile, short, plump rods, 0.6 by 1.0μ; facultative anaerobe, acid and gas produced on various carbohydrate media; no spores; capsules present; colonies on nutrient agar plates white, round, entire, glistening, opaque, some strains emitting a strong putrid odor; nitrates reduced to nitrites; ammonia produced; indol produced; hydrogen sulphide not produced (?); diastatic action marked; fair growth in Fermi's and Uschinsky's solutions; optimum temperature for growth 30°C.; vitality on culture media comparatively long; gelatin not liquefied; somewhat sensitive to drying, freezing, and sunlight; Gram negative; not acid fast. Pathogenic on *Zea mays* L.

SUMMARY

The bacterial stalk rot of corn has been reported from Arkansas, New York, North Carolina, Mississippi, Louisiana, Ohio, Illinois, North Dakota, and Arizona.

The disease has been noted in Arkansas for 6 years and is considered to be serious during those periods of the corn-growing season when the rainfall and humidity are above normal. In the absence of high temperatures and humidity the disease is found to be entirely absent or of minor importance.

[5] If one considers the aim of classification to consist primarily of bringing together and grouping into genera all forms that are morphologically related, then the recent proposals brought out by the Society of American Bacteriologists (1) will be condemned; but if the similarity of physiological processes, rather than morphology, be considered the guide, then the new proposals will be accepted. It is the humble opinion of the writer that so little is actually known about the morphology as well as physiology of the supposedly well known organisms, that almost any system which would be proposed is certain to be full of errors. The important thing for the present is agreement among bacteriologists on one system; otherwise, chaos in nomenclature is sure to rule. The recently proposed genus *Phytomonas,* therefore, is being accepted. If Migula's classification is followed, the combination should be *Bacterium dissolvens* (comb. nov.); and if Erwin F. Smith's classification is used, the combination *Aplanobacter dissolvens* (comb. nov.) is suitable.

It is described as primarily affecting the stalks and leaves. Symptoms are as follows: (a) a light- or dark-brown rotting of bases of leaves, particularly those at the base of the stalks; (b) a rotting of the lower portion of the stalk, the affected part being dark-brown, soft, putrid, and sunken in fresh infections. These may extend clear through the entire width; or, as more frequently found, remain localized as dark, rotted spots with margins appearing water-soaked. The disease consists of a localized necrosis of parenchymatous tissue.

Attacked tissue is found teeming with bacteria, and more than 50 isolations are reported from such infections. Many successful artificial infections are also reported, including infections obtained in Hottes chambers in which accurate control of soil and air temperatures are obtainable. It is found that temperature plays an important role in disease inception and development. Below 20° C. no infections take place; between 20° and 25° C. infections are few; between 30° and 35° C. the greatest number of infections result. High humidity is also found essential for infection. A correlation is noted between rapid succulent growth and disease susceptibility.

Infections are found to take place through hydathodes, stomata, and through injuries resulting from the extrusion of endogenous roots, and from insects.

The pathogene is described as a short, plump, rapidly growing rod, white in most culture media, and is considered to be closely related to *Bacillus coli*. Numerous cultural and physiological reactions are described. The pathogene is named *Phytomonas dissolvens* (comb. nov.).

AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY OF ARKANSAS.

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EXPLANATION OF PLATES

PLATE XVII

a—Natural infection at a lower node showing extreme necrosis and shrinking of diseased tissues and disjointed threads, representing remnants of the fibro-vascular bundles. Marked depression in nodal circumference due to destruction and collapse of parenchymatous tissue.

b—Artificial infections on Country Gentleman by means of needle pricks, the needle carrying pure cultures of the pathogene; infections appear as small, dark brown, or blackish streaks and spots.

c—Artificial infection on Mexican June field corn, in the form of a rotted internode, resulting from hypodermic injection of a pure culture of *Phytomonas dissolvens* (checks injected with sterile water remained healthy).

d—Natural infections on lower part of stalk, mostly in the form of localized spots.

e—Typical specimen, representing sheath infections, left by Dr. Burrill in the herbarium of the University of Illinois and labeled by him, "probably bacterial."

PLATE XVIII

a—Colonies of *Phytomonas dissolvens* isolated from a rotted node, 48 hours old, on nutrient agar. Surface colonies whitish, opaque, buried colonies are much flatter and appear lighter.

b—Growth on nutrient agar slant after 24 hours at 28° C.

c—Growth of the corn pathogene along entire length of stab in nutrient agar, with a gas bubble shown in one.

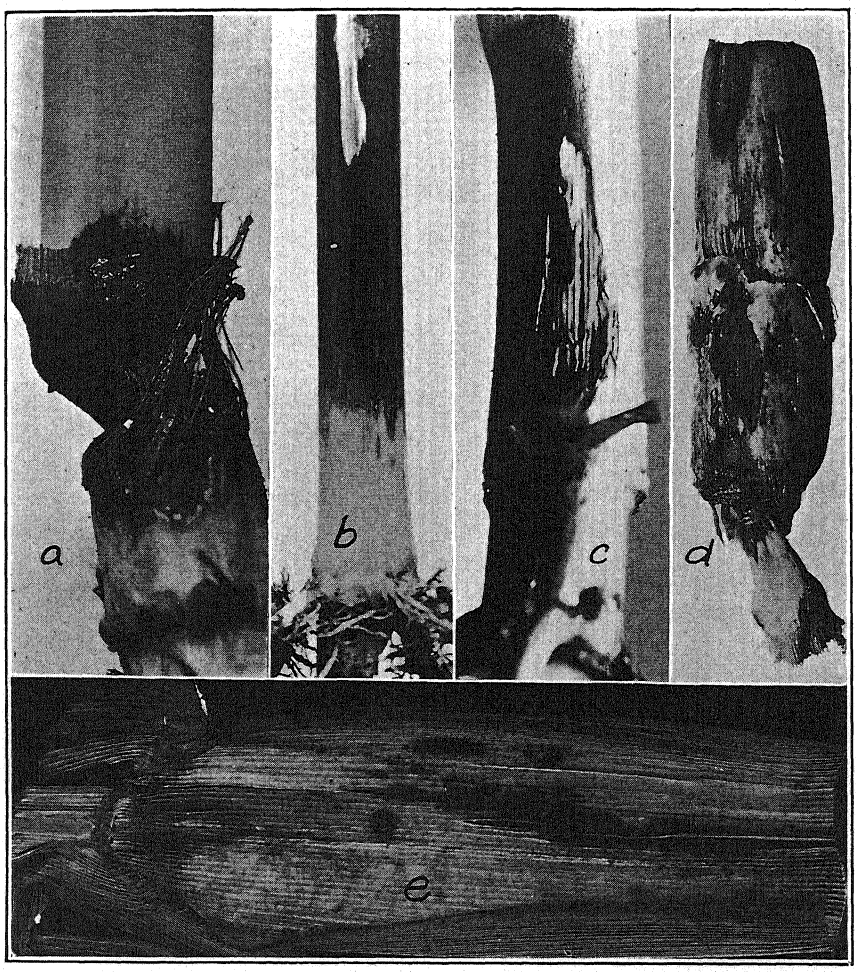
PLATE XIX

a—*P. dissolvens* stained by the Van Ermengem method for showing flagella. The streamer, connected to the rod on the extreme right, appears to be a flagellum, but, in view of the entire absence of motility in hanging drops of pure cultures, and in view of the extreme viscidity of certain strains, this and similar appearing structures are interpreted as being part of the slimy matrix surrounding the individual rods. Magnified about 1,000 times.

b—Artificial inoculation of *P. dissolvens* on Golden Bantam sweet corn, showing relationship of stomata to infection. View represents an unstained, water mount of a tangential section of the cornstalk immediately below the soil line. Note the bacteria, appearing as a dark mass, in a cell adjoining one of the stomata, the two other stomata and their neighboring cells being normal.

c—*P. dissolvens* stained with carbol-fuchsin. Magnified about 1,000 times.

d—Corn pathogene stained to show capsules. This staining enlarges the organism considerably as compared to ordinary staining. Magnified about 1,000 times.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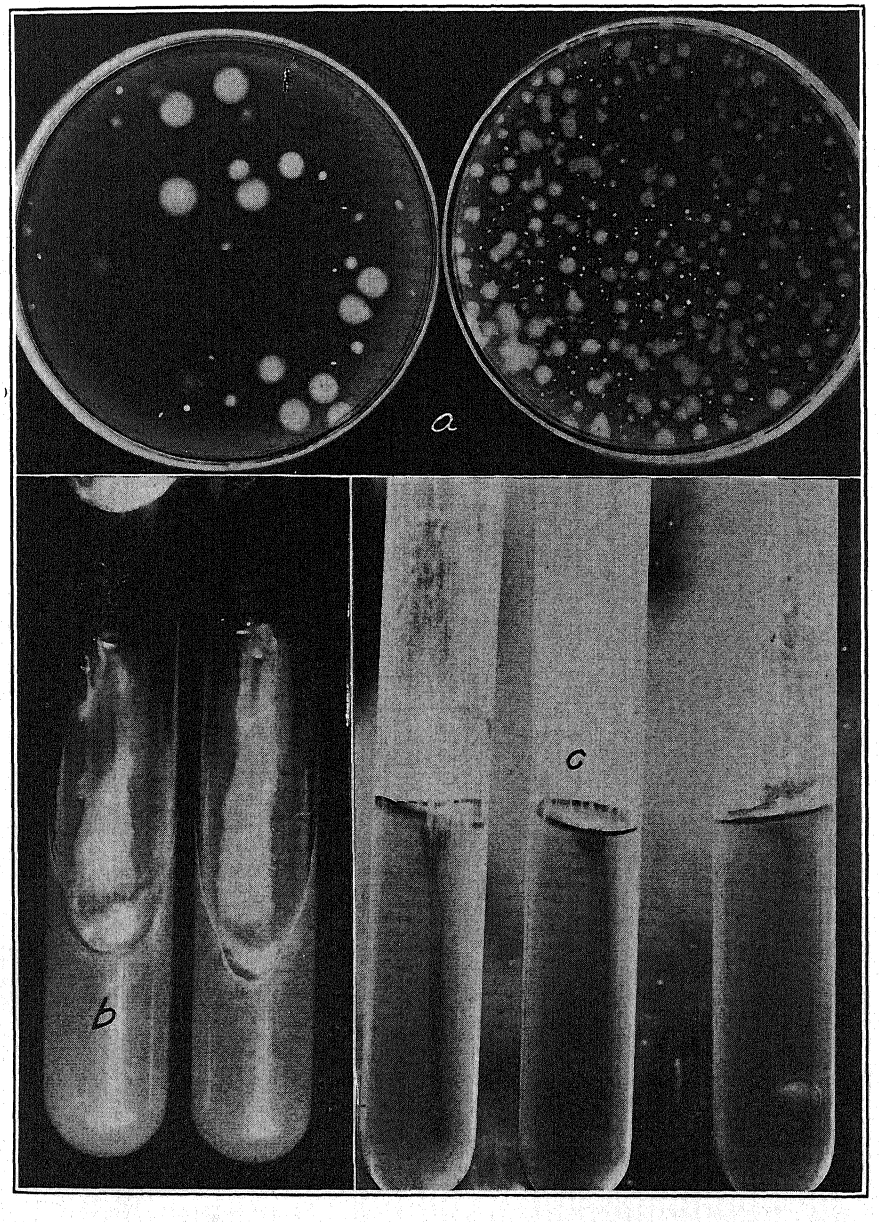
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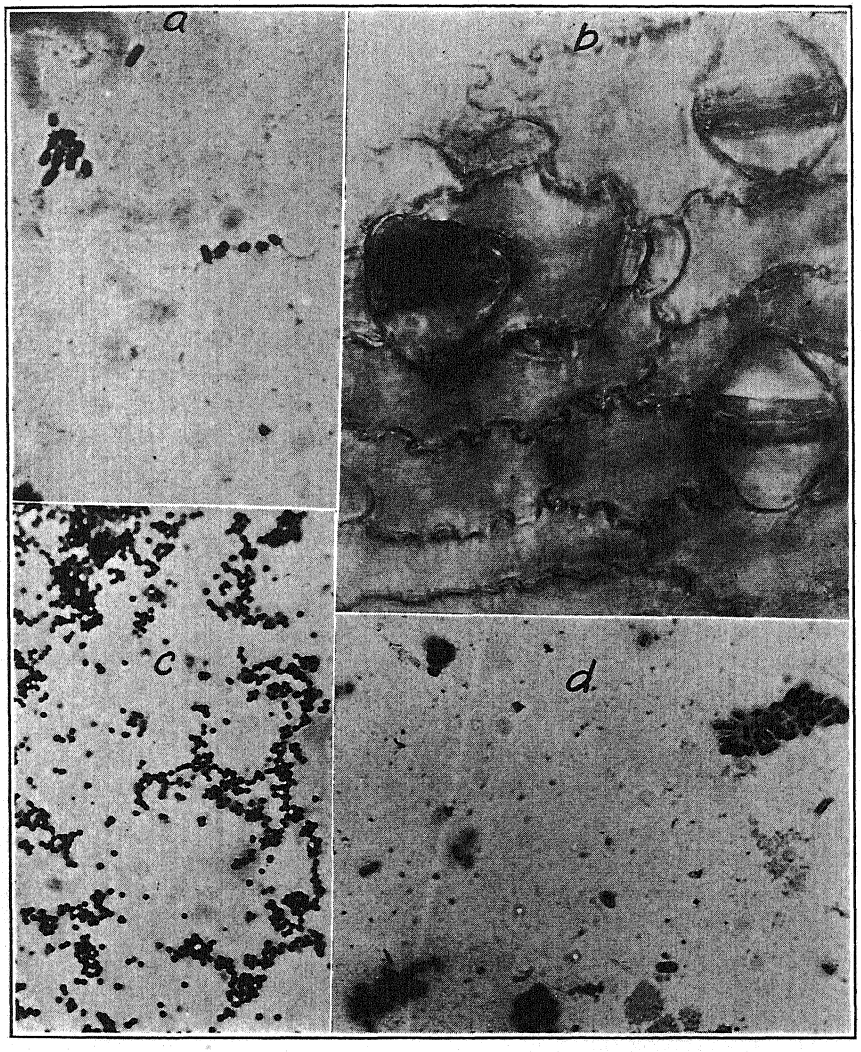
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THE NATURE OF RESISTANCE TO FOOTROT CAUSED BY ASCOCHYTA SP.[1] AND SOME OTHER FUNGI IN THE EPICOTYL OF THE PEA

GRACE G. GILCHRIST

INTRODUCTION

In the investigation by F. R. Jones (1) of the cause of failure of peas in certain parts of the United States, it was found that, in many dying plants, the base of the epicotyl and the upper portion of the taproot were decayed, sometimes to such an extent that the plant broke readily at this point. This type of injury is usually called footrot, to distinguish it from the more extensive decay of the root system called rootrot. Footrot, or decay of the base of the stem, is not due to a single parasite, but may be caused by any one of several widely different parasites upon the pea plant. Among these are *Fusarium martii* var. *pisi, Pythium* species, *Rhizoctonia solani* Kühn, and a species of *Ascochyta.* The fact that such a number of fungi find the base of the epicotyl, and even a limited region at the base of the epicotyl, more accessible to invasion than the upper portion of this underground structure, and more accessible than the taproot below, suggests that this region has some structural or other peculiarity which makes it easy of access to weak parasites. If the nature of this peculiarity can be discovered, and if it is found to be possessed unequally by different varieties of peas, making some more resistant to footrot than others, such finding will constitute a contribution to the studies of the nature of resistance of peas to disease, now in progress. Resistance of varieties of peas to soil inhabiting parasites which accumulate in old pea fields and which undoubtedly include those producing footrot seems to have been demonstrated by field trials in Wisconsin, as well as by several investigators elsewhere. It is the object of the present study to determine whether certain varieties resistant in field trials are resistant to the typical footrot caused by *Ascochyta* sp., and to discover the factor or factors which render the base of the epicotyl easy of access to this, and perhaps the other, parasites causing footrot.

[1] This is the "Phoma-like" fungus mentioned by Jones and Linford (2) as a cause of footrot of peas in Wisconsin. Further study of this fungus is in progress to determine its relation to *Ascochyta pisi* Lib.

DESCRIPTION OF FOOTROT CAUSED BY ASCOCHYTA

The fungus causing this disease enters the base of the epicotyl and from there spreads up and down through the cortical tissue. A dark brown lesion, triangular in shape, with the apex of the triangle pointing upward, is formed on the epicotyl. In slightly affected plants the fungus extends inward only as far as the endodermis, and there is no appreciable shrinking of the tissue. The fungus spreads downward into the taproot, but that organ is not usually seriously injured. In more severe cases the epicotyl is girdled, and there is considerable shrinkage of the tissues between the four strands of sclerenchyma, giving the injured stem an angular appearance. The taproot in this case is blackened, completely girdled, and may be severed. Here the vascular system is penetrated by the fungus. Pycnidia are found on these lesions and also on the seedcoat (Plate XX, C).

Lesions caused by this fungus are distinguished from those caused by *Fusarium martii* var. *pisi* chiefly by their color, those caused by *Fusarium* being somewhat of a reddish brown. Lesions caused by *Rhizoctonia* in this region of the epicotyl are usually more corroded and sunken in character than those caused by the *Ascochyta* (Plate XX). Lesions caused by *Pythium* species usually are sunken, but are straw colored or very light brown. Lesions produced by these fungi hardly can be distinguished by form or difference in location.

CHARACTERS OF THE EPICOTYL

The epicotyl, that portion of the stem between the point of attachment of the cotyledons and the first node, in the pea resembles the root in many respects. It produces both root-hairs and secondary roots. As a rule, however, these are formed, not at the very base of the epicotyl, but slightly above it. It is this hairless region which is most susceptible to attack by various parasites (Plate XX).

The true root possesses a definite endodermis, but immediately above the base of the epicotyl this endodermis is not entire, and, progressing further up, it becomes less and less conspicuous.

For detailed study of this region, material of the Alaska variety of pea was fixed in formal-alcohol (60 per cent alcohol—96 cc., formalin 4 cc.). Parts of the same plant, placed in the same fixative, gave varying results. Portions of the stem above soil level fixed excellently, whereas with the epicotyl the outer layers showed considerable plasmolysis, although the inner tissues were well fixed. It seemed as if the alcohol of the fixative had withdrawn water from the epidermal cells of the epicotyl more quickly than from the epidermal cells of the second or third internodes.

Transverse sections of this material stained with Sudan III showed marked differences in the amount of cuticle in the aerial portions of the

stem and in the epicotyl. In the epicotyl itself a diminution in the cuticular development was observed in passing towards its base. The difference in the amount of cutin between the top and the base of the epicotyl suggested that this might be a contributing factor in the resistance or susceptibility of various varieties of peas.

INFECTION RATING TO DETERMINE RELATIVE SUSCEPTIBILITY OF VARIETIES

Since differences responsible for resistance are more easily recognized in the comparison of similar varieties, representatives of similar growth maturing at different seasons were chosen. Alaska and Rice's No. 330 are both early varieties which mature at approximately the same time and are almost identical in appearance and habit of growth, the Rice's No. 330 differing primarily in its disease-resistant character. Saxa is also an early variety, which, owing to its great susceptibility to all pea diseases, has been discarded by commercial growers. Horsford and Horal are dwarf varieties, Horal being a Wisconsin cross between Alaska and Horsford, recently developed and introduced as a promising disease-resisting type. The White Marrowfat and Black-eye Marrowfat are old horticultural varieties and are used chiefly for garden or field culture. Future reference to the resistance or susceptibility of these varieties will be made by R. or S. These varieties were grown in the greenhouse in Wisconsin soil temperature tanks at approximately 18°C.[2] in soil which had been inoculated with the *Ascochyta*. The infection rating, according to a method described by McKinney (3), was as follows:

Susceptible varieties			Resistant varieties		
Name	Season	Per cent infection	Name	Season	Per cent infection
Saxa	early	64	Rice's No. 330	early	38
Alaska	early	42	Horal	mid-season	44
Horsford	mid-season	48	Black-eye Marrowfat	late	41
White Marrowfat	late	50			

MICROCHEMICAL TESTS

From the check plants in the above experiment, transverse sections were made of the epicotyl and stained with Sudan III for cutin. The resistant varieties, Rice's No. 330, Horal and Black-eye Marowfat, showed a definite

[2] Experiments conducted in the Wisconsin soil temperature tanks to determine the most suitable temperature for the early development of the pea plant showed that the greatest increase in weight took place at 18° C.

cuticle in the aerial parts of the stem. The reaction for cutin was less pronounced on passing towards the base of the epicotyl. However, at the level of the cotyledons, a slight amount was still present.

With susceptible varieties there was no appreciable difference in the cuticle of the aerial parts from that of the resistant varieties mentioned above, but, in passing towards the base of the epicotyl, the cuticle entirely disappeared.

This variation in the amount of cuticle present in the susceptible and resistant varieties suggested a possible difference in the rate of drying of corresponding portions of the epicotyl.

EXPERIMENTS TO DETERMINE THE RATE OF EVAPORATION FROM THE EPICOTYL

The above seven varieties were planted in sand, with soil immediately below them, and placed in a greenhouse with an air temperature of 18–20°C. At the end of seven days the plants were taken up, and the first internodes of twenty representative plants were selected. These were divided into three equal portions, base, middle and top. Records of the length and diameter of each portion were made. The latter measurement was read by means of a Zeiss micrometer gauge.

In order to avoid evaporation from the cut ends of the portions, these were dipped into melted vaseline. Weighings were made every two hours for eight hours. In calculating the per cent loss of water from the surface of each portion, it was necessary to take into consideration the weight of the vaseline. The latter was obtained by weighing a filter paper, heating it, allowing it to absorb the vaseline from the cut ends, and then re-weighing. The difference between these two weights gave the weight of the vaseline. From this data the actual weight of each portion was obtained.

Table I represents the loss of water calculated as a percentage of the original weight. It will be noted that in every variety tested there was a greater loss in weight from the base of the epicotyl than from the top of the epicotyl. Also, in comparing the similar varieties, there was a greater loss from the base of the epicotyl of Alaska than from that of Rice's No. 330; more from Horsford than from Horal; and more from White Marrowfat than from the Black-eye Marrowfat. From these results, together with the area of the evaporating surface calculated from the measured length and diameter of each portion, the amount of water evaporated from 100 sq. cm. was calculated. The results thus obtained are shown graphically in figure 1. They show that there was a direct correlation between the rate of evaporation and relative susceptibility to footrot among similar varieties. There was more evaporation from Alaska (S) than from Rice's No. 330 (R), more from Horsford (S) than from Horal (R), and more from White Marrowfat (S) than from Black-eye Marrowfat (R).

DISCUSSION

Several points of interest may be deduced from these results. In all the varieties examined, more moisture is evaporated from the surface of the lower third of the epicotyl than from the upper third. This result

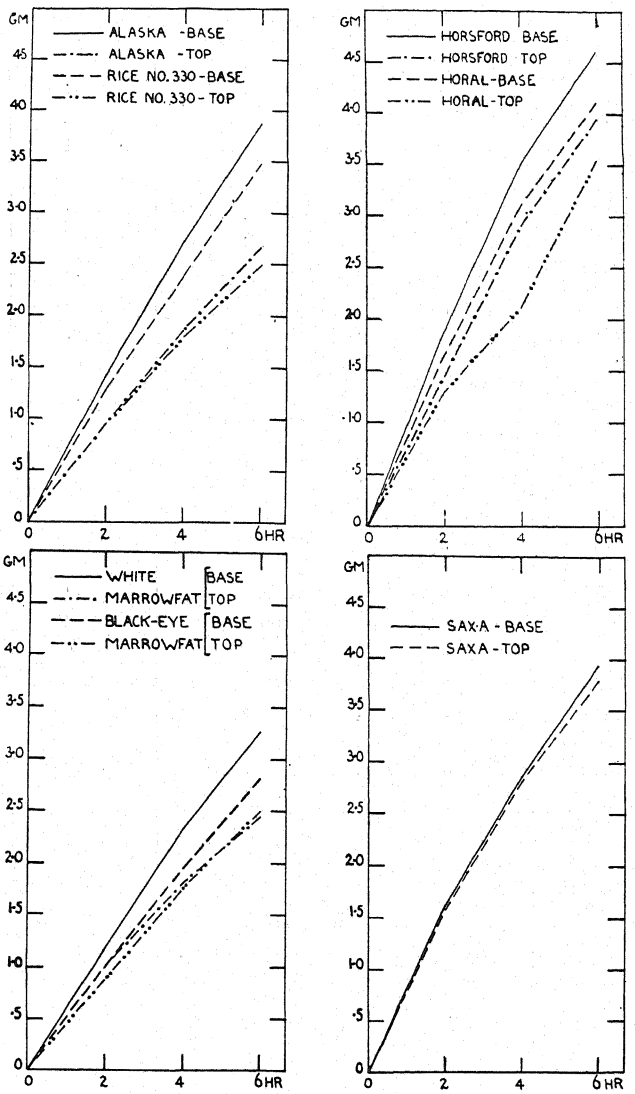

FIG. 1. Graphs representing the loss of water by evaporation at intervals of two hours. A direct correlation between rate of evaporation and susceptibility to pea footrot is shown. The rate of evaporation increases with the increased susceptibility of varieties and of the different portions of the epicotyl.

TABLE 1.—*Summarized data of the rates of evaporation of different portions of the stem on the same pea plant and also the rates shown by plants of varieties differing in relative susceptibility to fungous invasion*[a]

Variety	Water loss in per cent			
	After 2 hours	After 4 hours	After 6 hours	After 8 hours
Rice's No. 330 (R)				
Base	22.3	41.5	60.1	74.3
Middle	21.0	37.0	52.3	64.2
Top	16.7	31.0	44.1	52.0
Alaska (S)				
Base	27.3	50.9	72.4	85.5
Middle	20.4	39.6	54.6	64.1
Top	17.5	34.1	49.0	60.2
Horal (R)				
Base	25.1	46.7	58.1	77.8
Middle	27.7	51.8	69.6	80.4
Top	18.1	29.5	49.3	61.4
Horsford (S)				
Base	32.6	60.0	79.7	88.9
Middle	27.1	51.5	71.9	84.6
Top	23.2	44.4	62.8	77.1
Black-eye Marrowfat (R)				
Base	16.8	32.7	47.0	59.2
Middle	16.5	30.1	43.9	54.2
Top	13.9	28.0	39.7	50.3
White Marrowfat (S)				
Base	20.1	39.9	56.1	70.7
Middle	18.1	33.2	47.5	60.6
Top	16.4	29.3	40.0	50.0
Saxa (S)				
Base	28.4	52.4	69.9	80.5
Middle	29.3	52.4	69.6	78.2
Top	26.5	48.0	64.4	74.8

[a] Note that in all cases the rate of evaporation increases from the more heavily cuticularized top to the less heavily cuticularized base. Note also, in comparing the relatively susceptible (S) and resistant (R) varieties as paired, that there is a direct correlation between higher rate of evaporation and the relative susceptibility of the variety.

corresponds with that obtained by microchemical tests, and seems to point to the fact that the lower portion of the epicotyl is deficient in cuticle. Since cuticle consists of an unbroken lamella containing no cellulose fabric,

it forms an effective barrier against the penetration of most fungi. Consequently the lack or deficiency of it at the base of the epicotyl of the pea may account for the susceptibility of that region to numerous fungi. Again comparing Rice's No. 330 and Alaska, both early season varieties, the loss of water after two hours from Rice's No. 330 (R) was 22.3 per cent as compared with 27.3 per cent from Alaska (S); in the mid-season varieties, the loss from Horal (R) was 25.1 per cent as compared with 32.6 per cent from Horsford (S); and in the late-season varieties, the loss from Black-eye Marrowfat (R) was 16.8 per cent as compared with 20.1 per cent from White Marrowfat (S). Whether the presence or absence of cuticle is entirely responsible for susceptibility or resistance is a difficult question to answer, but the above results clearly seem to suggest that there may be an important correlation. No variety of pea has been found to be immune to footrot, but those which are more resistant possess a thicker cuticle than those which are less resistant. The base is the most susceptible portion of the epicotyl, and it is this portion which develops the thinnest cuticle. Priestley (4) states that the difference between cutin of different plants is due in part to differences in their constituent acids, and in part to differences in the external and internal conditions prevailing whilst these acids pass over into the form that they assume in the mature cutin lamella. Therefore the above results are relative, and should not be regarded as absolute. Consequently, should these experiments be repeated, the amount of water evaporated from the same varieties probably would not coincide with the above results, but the relative amount should be comparable.

In the case of the Ascochyta footrot in plants which have been attacked but recovered, the fungus appears to have penetrated at the base of the epicotyl and traveled inwards as far as the endodermis. Here it seems to have met a barrier comparable with that of the cuticle on the surface. Therefore an investigation of the endodermis in the pea plant as to its possible relation to disease resistance should give further interesting results.

SUMMARY

1. In previous investigation of the diseases of the garden pea, it has been observed that a group of parasitic fungi enter the base of the epicotyl more frequently than elsewhere in the underground stem or root. These fungi include a species of *Ascochyta* which causes a footrot of peas in Wisconsin. This paper presents the results of a search for the characteristics of this basal portion of the epicotyl which render it especially susceptible to invasion, and for possible differences in susceptibility of this region in different varieties of peas.

2. Microscopic observation and experiments on rate of evaporation have shown that the susceptible region at the base of the epicotyl is deficient or

lacking in a covering of cuticle. The middle and upper portions of the first internode show a thicker cuticle in all the varieties studied.

3. Inoculations with a species of *Ascochyta* have shown that varieties of peas described as resistant in field trials are less injured by this fungus than are the susceptible varieties. Comparison of these varieties has shown that the resistant varieties have a better development of cuticle at the base of the epicotyl than do the susceptible ones.

4. From this work it appears that deficiency or absence of cuticle at the base of the epicotyl is one of the conditions which make this region particularly susceptible to disease of the footrot type, and that varietal difference in susceptibility may be due in part to differences in the degree of cuticularization of this region.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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DESCRIPTION OF PLATE XX

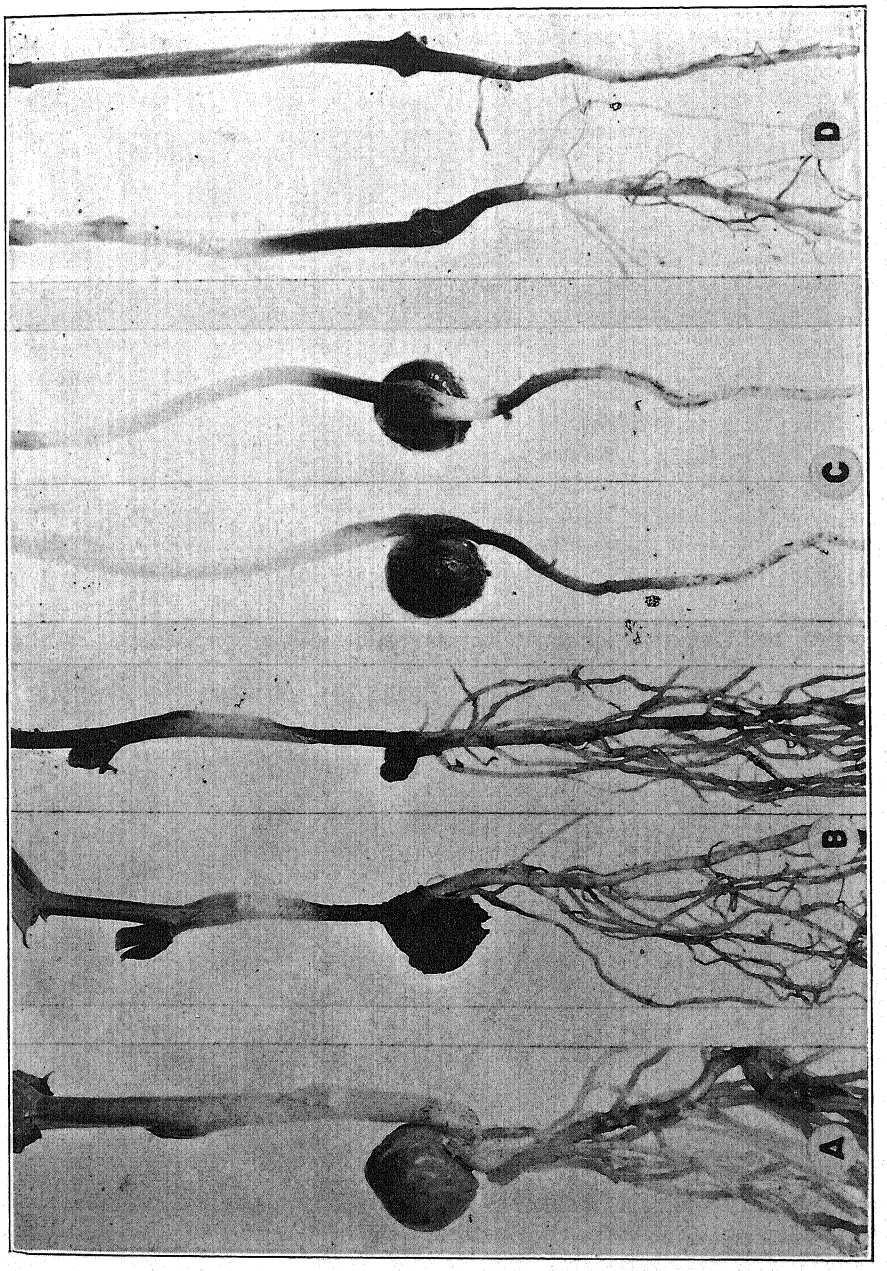
Photographs of pea plants showing lesions at the base of the epicotyl caused by *Rhizoctonia solani, Ascochyta* sp. and *Fusarium martii* var. *pisi*.

A. Healthy pea plant.

B. Pea plants attacked by *Rhizoctonia solani* in the field.

C. Pea plants attacked by *Ascochyta* sp.—grown in inoculated soil in the greenhouse.

D. Pea plants attacked by *Fusarium martii* var. *pisi*—grown in inoculated soil in the greenhouse.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LITTLE LEAF DISEASE OF PECANS[1]

J. B. DEMAREE

INTRODUCTION

A disease of the pecan, here provisionally named "little-leaf,"[2] which is widely different from the commonly known diseases of this tree, was first observed by the writer in Ocean Springs, Miss., in 1920. The following year, pecan trees affected with the same disease were found in Gainesville, Fla. In 1924 the disease was observed on trees growing in yards in Biloxi, Miss. In addition to the diseased trees seen by the writer, affected leaves have been received from Darien and Savannah, Ga. Reports of "little-leaf" have come from two sources in Savannah. Dr. O. F. Burger, University of Florida, reports that he saw a pecan tree affected with this disease in Miami, Fla.; and Dr. L. E. Miles, A. & M. College, Auburn, Ala., has informed the writer of the occurrence of "little-leaf" on a pecan tree at Troy, Ala. Matz[3] undoubtedly observed the same trouble in Gainesville, Fla., in 1917. In his bulletin he illustrated what appears to be a typical case of pecan "little-leaf."

DESCRIPTION

A normal pecan leaf is composed of from 11 to 15 leaflets and varies from 8 to 20 inches in length. The leaflets are falcate, oblong-lanceolate, serrate, and range from 3 to 6 inches in length. Leaves affected with the "little-leaf," as a rule, are composed of a reduced number of leaflets, occasionally as few as two (fig. 1) and averaging 8 or 9 to the leaf. The terminal leaflet is often absent on affected leaves, giving them an even number of leaflets. The shape of an abnormal leaflet varies from almost round to oblong, and lacks the sickleshaped and pointed apex, which is very characteristic of the normal leaflet. The apical portion is often dead, dry, and ragged. The serrations of diseased leaflets are also less pronounced than those of normal leaflets. In more accentuated cases, the leaflets are both shortened and narrowed, the width being reduced to about one-third inch. The leaf rachis is also reduced in length, and many leaves are only one to

[1] Paper read before Southern Section, American Phytopathological Society, meeting with the Association of Southern Agricultural Workers, Atlanta, Ga., Feb. 3, 1925.

[2] The disease is known locally in south Mississippi as "mouse-ear."

[3] Matz, J. Diseases and insect pests of pecans. Fla. Agr. Exp. Sta., Bul. 147: 135–163. 1918.

FIG. 1. Pecan leaves affected with the "little-leaf" disease. Note size and shape of leaflets in comparison with normal (lower) leaflet. Natural size.

two inches long, exclusive of the petiole. A slightly affected leaflet may be normal in size, the only visible symptom of the disease being the obtuse apex.

Badly affected twigs are extremely short and slender, with internodes correspondingly shortened (fig. 2). Buds on affected twigs are weak, undersized, and bluntly pointed. Those near the terminal end of the twig fail to develop. The general appearance of diseased buds and their frequent failure to resume growth the following spring indicate they are lacking in vitality.

Some trees observed were so severely affected that all leaves produced on the trees were composed of leaflets of the extremely rounded and diminutive type. The disease in other trees may be found only on certain branches and will recur each year on the same limbs, other limbs of the same trees producing normal leaves and twigs. Badly affected trees do not bear nuts, and their growth is greatly retarded. The writer has evidence that some trees recover after one or more years, while it is definitely known that the trouble has persisted in others for a number of years.[4]

DISTRIBUTION

It is remarkable that the disease is not known to occur under orchard conditions, but all cases so far observed have been in trees growing in gardens and lawns within city limits, and, with one exception (Troy, Ala.), confined to localities close to the Atlantic or Gulf Coasts. While no extensive study has been undertaken to locate the distribution of the "little leaf disease" of the pecan, in a general way it may be said to be present from Savannah, Ga., south to Miami, Fla., and west to Biloxi, Miss. It is believed that the disease will later be found in many other towns, especially those situated near the Atlantic and Gulf Coasts. A larger number of affected trees are known to be in Ocean Springs, Miss., and Gainesville, Fla., than at other points.

Hodgson[5] reports a "little leaf" disease of deciduous fruit trees as being prevalent in California. This western type of "little leaf" is characterized by the failure of the trees to leaf out at the normal time and, when they finally are formed, they are very small and narrow, and yellow in color. This condition may involve the entire tree or it may be confined to one or two limbs only. The appearance and effect varies somewhat on different kinds of fruit trees. In the peach, this disease is characterized by

[4] According to Mr. Theo. Bechtel, pecan grower and nurseryman, Ocean Springs, Miss., one tree at least has been known to have been affected continuously for the past fifteen years.

[5] Hodgson, Robert W. "Little leaf" of deciduous fruits. Mo. Bul. Calif. State Comm. of Hort. 7: 529–532. 1918.

the production of long, slender shoots on the new growth, terminated by a cluster or tuft of leaves. The small leaves along the branch usually are shed relatively early in the season. In the apple and pear, "little leaf" usually manifests itself more in the dwarfing of the leaves on the affected

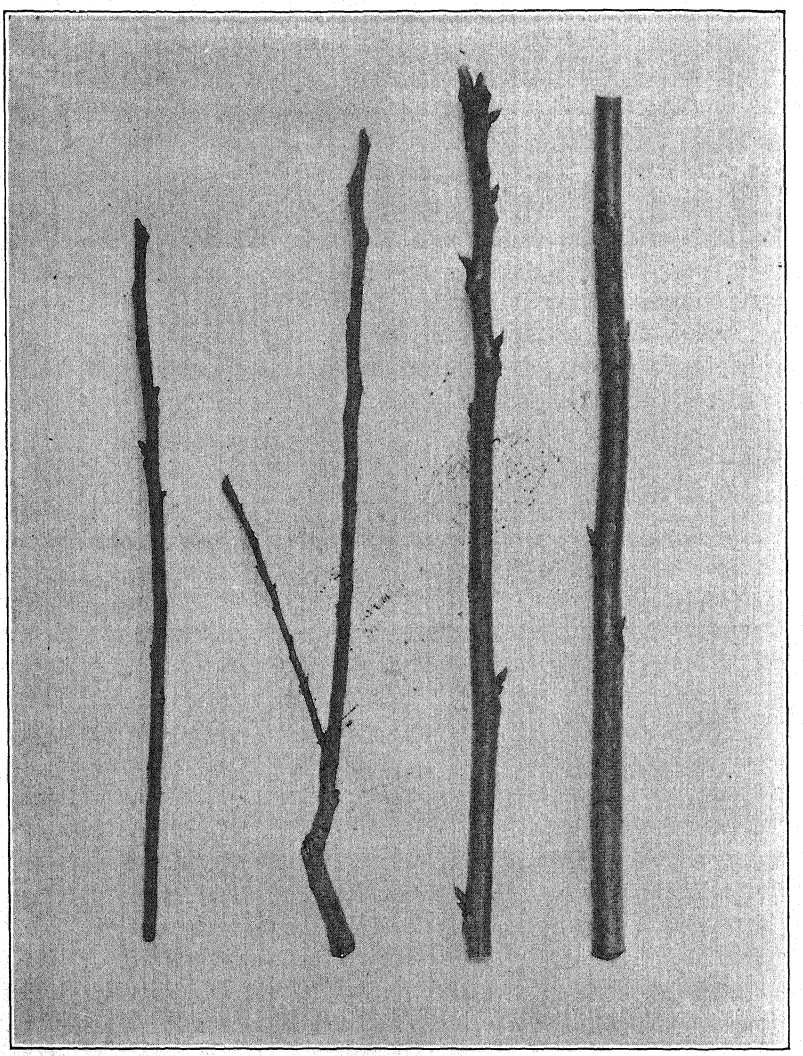

FIG. 2. Photograph of pecan twigs. Two on left affected with "little-leaf." Two normal twigs on right. ⅓ nat. size.

branches. In the walnut, the effect consists usually in greatly delayed leafing out, the resulting leaves being small and decidedly mottled in appearance.

The cause of "little leaf" of deciduous fruit trees in California is attributed to unseasonable climatic conditions, such as long, dry winters. There is a decided correlation, as reported by Hodgson, between the presence of the California "little leaf" and light sandy or arid soil.

PROBABLE CAUSE OF THE DISEASE

Very little is known of the factor or factors which induce the development of pecan "little-leaf." The temperature, rainfall, soil, and other environmental conditions are quite similar at all points where the disease is known or reported to be present. There has been no correlation observed between its prevalence and a deficiency of soil moisture, as is reported in the case of the California "little leaf" disease.

There is no indication that the disease is transmissible, except that in two towns, Ocean Springs, Miss., and Gainesville, Fla., where the disease has been known to be present for a number of years, an unusually large number of diseased trees have been found. Two or three diseased trees are sometimes found on the same lot, garden, or lawn, but, in such cases, some condition of the soil may be the predisposing factor. Healthy trees are known to have stood within a distance of 50 feet of a badly affected tree for a period of from 12 to 15 years without showing any indication of infection.

As the factor causing the diminutive leaves and slender twigs was thought by some growers to be in the branches, pruning the affected trees severely and burning the severed parts were tried in an effort to eradicate the disease. No favorable results were obtained: the new leaves growing from the stubs were diseased also.

Matz[6] attributes the abnormal leaves to low temperature injury to the buds after they begin to swell. The writer has not observed any correlation between this condition of pecan leaves and the weather, and does not believe that the trouble is caused by late frosts, as suggested by Matz. A leaf trouble caused by frost injury would necessarily only affect those leaves appearing during early spring. But when a tree is badly affected with "little leaf," even those leaves that are formed at the ends of growing twigs during mid-summer may have the reduced number of small round or oblong leaflets so characteristic of the disease. Then, too, frost would not likely cause this type of trouble in the same trees annually over a period of from twelve to fifteen years. The possibility of frost injuring the leaf buds on one or two limbs of a tree would be remote. Since the trouble has been found only in trees growing in the extremely fertile soils usually prevalent in old established lawns and vegetable gardens, it may be due to the action of some injurious soil substance there.

[6] Matz, J. *Ibid.*

EXPERIMENTAL WORK

The question arose as to whether the disease could be disseminated by using diseased scions or buds in propagation of nursery trees. During February, 1923, 24 scions taken from a badly diseased tree in Ocean Springs, Miss., were grafted on one-year-old nursery seedlings at Thomasville, Ga. Those twigs used did not have the vigorous, plump appearance of normal twigs. They had more the appearance of short, slender switches than vigorous, promising scions. The contrast is well illustrated in fig. 2. Although the grafting was done by an experienced nurseryman of many years' success, and special care was taken in placing and tying the scions on the stock, only 3 of the 24 lived. The growth resulting from these 3 living scions was normal in every respect.

In July, 1923, an attempt was made to transmit the disease by budding. Twelve buds taken from diseased twigs were inserted in the twigs of young healthy trees. All of the buds except two died, and these produced normal vigorous branches.

In April, 1924, 25 scions from a tree affected with "little leaf" were cleft-grafted in healthy five-year-old trees. Remembering the failure of slender switch-like scions in the year previous, more vigorous appearing twigs were used. Those were selected, however, that showed signs of disease as evidenced by the small blunt buds. As a result of this trial, 9 lived and produced healthy branches, while the remainder failed to grow. It is also worth while to record here that the contact of the scions or buds with the stock did not in any instance transmit the disease-producing element to the stock. While the attempts that have been made to transmit the disease to healthy trees cannot be considered as conclusive on account of the limited number of trials made, the evidence suggests that the disease is not likely to be transmitted by the ordinary nursery propagating methods. During February, 1924, a six-year-old pecan tree located at Ocean Springs, Miss., that had been badly affected with "little-leaf" for three years previous, was transferred by the writer's request to a point outside of the city and replanted in an Orangeburg type of soil in an environment approximating field conditions. The tree continued to live and showed slight symptoms of "little leaf" during the season of 1924. The tree was again kept under observation during the season of 1925, and not the slightest trace of the disease could be found.

SUMMARY

A disease affecting the leaves and twigs of the pecan tree has been under observation since 1920 and is here named "little leaf."

Affected leaves, as a rule, are composed of a reduced number of leaflets, averaging about eight to the leaf and sometimes reduced to two. Affected

leaflets are oblong or rounded, often with obtuse or notched apices, and frequently are greatly reduced in size. Affected twigs are slender, lacking in vitality, and the buds are small and blunt.

This condition of pecan trees has been found in Savannah and Darien, Ga., Gainesville and Miami, Fla., Troy, Ala., and Ocean Springs and Biloxi, Miss. The disease has been found only within city limits and never under orchard conditions.

Unsuccessful attempts were made to transmit the disease by budding and grafting. A badly affected tree transplanted to a location approximating field conditions entirely recovered.

The cause of the disease is not definitely known.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY,
THOMASVILLE, GA.

RELATION OF OXYGEN TO SPORE GERMINATION IN SOME SPECIES OF THE PERONOSPORALES

B. N. UPPAL[1]

INTRODUCTION

Oxygen is considered one of the chief requisites in the process of germination. It is well known that the seed of the phanerogam will not germinate if the requisite supply of oxygen is not available, even though suitable conditions of moisture and temperature prevail. It is also generally agreed that, in fungi where spores germinate by the production of a germ tube, the relation of oxygen to germination is essentially the same as in a germinating seed. However, the question arises, in how far does there exist in the conidia of the species of *Phytophthora* a physiological similarity in regard to the processes of direct and indirect germination, in relation to the requirements of oxygen? In other words, are we to consider the formation of zoospores as a process physiologically similar to that of the production of germ tubes? Moreover, in how far is the relation of oxygen to indirect germination a characteristic of other forms of the Peronosporales?

Ward (5) considered that the liberation of zoospores was favored by the presence of free oxygen. Melhus (2) concluded that there was sufficient oxygen in the conidium of *Phytophthora infestans* to permit germination without the presence of free oxygen in water. Murphy (3) observed that the presence of yeasts and bacteria in culture arrested germination of the conidia of *Phytophthora infestans.* He seemed to think that the competing organisms used up oxygen in the water, thus retarding germination of the sporangia. He used various organisms to contaminate the cultures, and, judging from the similarity of results obtained, inclined to the view that the question of excretory products was not involved. The conflicting evidence as to the role of oxygen in indirect germination suggested that it might be profitable to attack this problem anew.

TRIALS IN WHICH THE AIR WAS REMOVED WITH A VACUUM PUMP

A series of experiments was planned to determine whether or not the conidia of *Phytophthora colocasiae*[2] germinate in the absence of oxygen in

[1] The writer takes great pleasure in expressing his gratitude to Professor I. E. Melhus, under whom this work was done, for the many valuable suggestions and helpful criticisms given during the progress of this work.

[2] My sincere thanks are due to Dr. William MacRae, Imperial Mycologist to the Government of India, and to Dr. N. J. Giddings, of the West Virginia Station, for supplying me with cultures of the species of *Phytophthora.*

the surrounding atmosphere. The first trials were made in an atmosphere from which the air had been exhausted by means of a vacuum pump. Microscopic glass culture slides, one by three inches, with two polished circular concavities, each measuring 18 mm. in diameter and approximately 1.75 mm. in depth, were used for growing cultures. Double distilled water was boiled to drive out any absorbed air, and cooled rapidly to the temperature of the room. The concavities on the slides were filled with the distilled, cooled water, to which the spores were then added. The culture slides were placed in a desiccator over 200 cc. of boiled water.

The desiccator was connected to a vacuum pump and exhausted, until the air within it had been so much attenuated that it could hardly support a six mm. mercury column. Exhaustion of the desiccator was continued for another five minutes. In a short time the water at the bottom of the desiccator, becoming agitated briskly, was changed rapidly into water vapor, producing a misty atmosphere within. This water vapor helped to wash out the last traces of air in the desiccator, in which the vapor pressure was 19.630 mm. of mercury at 22° C.

The desiccator was placed outside on a window sill, on a wet, cool (10–15° C.) night. Checks were made similarly in another desiccator, except that no air was removed. At the end of two hours none of the spores in the oxygen-free desiccator had germinated, while those in the checks had germinated profusely and zoospores were still being formed.

The following day the same experiment was repeated. The desiccators were kept in a compartment of an automatically controlled refrigerator, registering 11–12 ° C., and the cultures were examined after seven hours. Careful examination of the cultures in the oxygen-free desiccator revealed a few empty conidia but only one or two zoospores; probably the rest had disintegrated.

At the end of the experiment the cultures to which a few drops of water were added (since they had almost dried up) were placed on a table at room temperature (22° C.) for 30 minutes. They were examined after this period, and many of the spores germinated and produced zoospores. Evidently, then, the conidia were not injured and had retained their power of germination.

It was observed in the experiments just described that, after an exposure of one hour or so to conditions within the desiccators, the culture drops had almost dried up. Naturally the idea suggested itself that this drying of the culture drops may have had something to do with the inability of the conidia to germinate in an oxygen-free atmosphere. To obviate this difficulty, syracuse watch glasses, about two and one-half inches in diameter, were used in place of microscopic culture slides.

As in the previous experiments, double distilled water was boiled, rapidly cooled, poured into the syracuse watch glass to fill one-third of its volume and charged with the spores. The rest of the treatment was similar to that described in the previous experiments, except that two desiccators were used, one of which was opened for examination after seven hours and the other after nine hours.

When the cultures in the oxygen-free desiccators were examined zoospores were abundant, a few of which were still moving about rather sluggishly. After about seven minutes at room temperature (24° C.), most of the ungerminated conidia in the cultures had started forming zoospores.

The following day the same experiment was repeated, but when the vacuum pump had so much attenuated the air within the desiccators that there was barely enough pressure to support a five mm. mercury column, exhaustion of one desiccator was continued for 12 minutes and that of the other for 25 minutes, after which they were stored in a refrigerator at 11–12° C. for six hours. When examined after this period, almost 100 per cent of the conidia had germinated by zoospores. The experiment was repeated with similar results.

TRIALS IN WHICH OXYGEN WAS REMOVED FROM THE AIR IN THE DESICCATOR

Although there was no doubt that exhaustion of the air from the desiccators was as complete as could be obtained under the conditions of the experiment, it seemed advisable to remove the oxygen with an alkaline solution of pyrogallic acid.

In two trials two desiccators were set up with the spores of *Phytophthora colocasiae* sown in boiled, distilled water in syracuse watch glasses. For the elimination of oxygen from these desiccators, an alkaline solution of pyrogallol was used, which was prepared in the two following ways: first, a solution of one part of pyrogallol in three parts of water was mixed with an equal volume of a solution of one part of potassium hydroxide in three parts of water. This solution, when freshly prepared, absorbs 10 times its volume of oxygen, and has been designated as Berthelot's solution of pyrogallol (6). Second, a solution was made with one gram of pyrogallol and 100 cc. of 10 per cent potash-water for every 100 cc. of air space. This solution will be referred to in this paper as Mace's solution of pyrogallol (4).

The desiccator receiving Berthelot's solution of pyrogallol had enough reagent to absorb one and three-quarters times the volume of oxygen that was actually present in the air of the desiccator. The other desiccator which received Mace's solution of pyrogallol had enough of the solution to absorb one and one-half times the volume of oxygen present in the air of the desiccator. Both the desiccators were allowed to stand at room tempera-

ture (24° C.) for 30 minutes before they were removed to a refrigerator at 11–12° C., for the absorption of oxygen is very slow at temperatures below 15° C., and it is also believed that when oxygen is absorbed slowly a large amount of carbon monoxide is produced (4). In this connection the author has made a number of experiments and has found that when desiccators containing an alkaline solution of pyrogallic acid were removed immediately to a refrigerator at 11–12° C. and allowed to stand there for from two to six hours, the gas inside the desiccators, tested by Orsat Gas Analysis Apparatus, usually showed from zero to two per cent of the volume of oxygen absorbed as carbon monoxide (1).

The cultures from both the desiccators were examined after six hours, and approximately 100 per cent of the conidia had germinated to produce zoospores. Checks gave similar results.

In another series of experiments two desiccators were set up, both receiving Berthelot's solution of pyrogallol for the elimination of oxygen. The reagent used was enough to absorb twice the volume of oxygen actually present in each desiccator. This amount was used as a precaution against accident. The desiccators were allowed to stand at room temperature (23° C.) for 30 minutes. One of the desiccators received no other treatment and was removed to a refrigerator at 11–12° C.; the other was connected to a vacuum pump and exhausted, until there was brisk agitation in the alkaline solution of pyrogallol in the bottom of the desiccator. The desiccator was then removed to the refrigerator. Examination of the cultures after six hours showed zoospores as abundant as in the check.

Another set of experiments was planned on the basis of the following hypothesis. If oxygen is essential for growth (as is generally accepted), and if direct germination is a form of growth, conidia of *Phytophthora colocasiae* in an oxygen-free atmosphere and at 29° C., or at higher temperatures, should not germinate by tube.

Two desiccators were set up, both receiving Berthelot's solution of pyrogallol for the elimination of oxygen. The reagent used was enough to absorb twice the volume of oxygen actually present in each desiccator. After standing at room temperature (22° C.) for 20 minutes, one desiccator received no other treatment and was removed to a thermo-electric incubator, in which the temperature was maintained at 30–31° C. The other desiccator was connected to a vacuum pump, the air exhausted in the usual manner, and the desiccator removed to the thermo-electric incubator.

The desiccator from which the air was not exhausted but had received the alkaline solution of pyrogallol was incubated for 12 hours. Upon examination it was found that the conidia in these cultures had not germinated during this period. The other desiccator, from which the air was exhausted, was incubated for 22 hours. Even in this case none of the conidia germi-

nated. In all checks the conidia germinated profusely by means of germ tubes. Direct germination, therefore, seems to require oxygen in the air around the spores. The experiment was repeated three times, with the difference that all cultures were examined after 24 hours. None of the conidia germinated, although checks always germinated well.

EXPERIMENTS WITH SOME OTHER SPECIES OF PHYTOPHTHORA

After having found that indirect germination in *Phytophthora colocasiae* was not dependent upon free oxygen in water, an attempt was made to ascertain whether the same relationship holds in the other species of *Phytophthora.* Three additional species, *Phytophthora infestans, P. palmivora,* and *P. parasitica,* therefore were used. *Phytophthora infestans* and *P. palmivora* were grown on oatmeal agar, the former also being grown on cut potato tubers. *Phytophthora parasitica* was cultivated on cornmeal, which was prepared by adding to the meal sufficient water to keep the medium moist after sterilization.

As in the experiments above described, Berthelot's solution of pyrogallol was used to remove oxygen from the desiccators, which were then allowed to stand at room temperature for 20 minutes before they were connected to an electric vacuum pump to exhaust the remaining air. Exhaustion of the desiccators was continued for 10 to 15 minutes after the air had been so much attenuated that it could hardly support a six mm. mercury column. The cultures were incubated at 11–12° C. for 18 hours. Examination after this period showed that zoospores had formed in all cultures in an oxygen-free atmosphere. All checks germinated well; the percentage of germination in cultures of *Phytophthora infestans* was about 50 per cent. These experiments were repeated three times at three different periods with essentially similar results.

In another series of experiments with the three species of *Phytophthora,* Berthelot's solution of pyrogallol was used to remove oxygen from the desiccators, which, however, were not exhausted by a vacuum pump. The results on germination were the same as in the previous experiment.

EXPERIMENTS WITH SOME SPECIES OF PLASMOPARA, PERONOSPORA, SCLEROSPORA AND ALBUGO

The peculiar response of indirect germination of the conidia of the species of *Phytophthora* to lack of free oxygen around the cultures was so interesting that it was deemed desirable to extend these observations to spore germination in the other species of the Peronosporales. Spore germination in *Albugo, Plasmopara, Peronospora* and *Sclerospora,* was studied.

The method of removing oxygen from the desiccators, in which cultures were placed, was the same as that employed in the above experiments. The

conidia of *Sclerospora graminicola, Plasmopara viticola* and *Albugo candida* failed to germinate in cultures surrounded by an oxygen-free atmosphere. The checks always germinated well, except that in *Albugo candida* germination of the conidia varied greatly, and was always less than 50 per cent. The conidia of *Peronospora trifoliorum,* which germinate by the production of germ tubes, also failed to germinate in the oxygen-free desiccators.

DISCUSSION

The experimental data above presented seem to indicate the following relationships of oxygen to spore germination in the Peronosporales: 1) except in *Phytophthora,* direct and indirect germination of the spores of the species of the Peronosporales is dependent upon free oxygen, other conditions for germination being optimum; 2) in *Phytophthora* indirect germination is more or less a result of the effect of temperature, and is independent of the relation of oxygen, at least in so far as the atmospheric oxygen is concerned; and 3) direct and indirect germination are two different phenomena. The former is a type of growth, while the latter is nothing but the breaking up of the intersporangial protoplasm aroused to activity by the invigorating influence of temperature, into independent protoplasmic units, each unit being endowed with the same power of direct germination as is a conidium under certain conditions.

That indirect germination in *Phytophthora* is independent of the relation of free oxygen is contrary to the observations of Ward (5), Murphy (3), and others, who claimed for oxygen a stimulating influence upon this type of germination. However, the contention of Melhus (2) that "free oxygen in the water is unnecessary for germination, and . . . the conidium contains within itself sufficient oxygen for germination," seems worthy of examination. Granting that there is sufficient oxygen within the sporangium to insure its germination when placed in water and under the right conditions of temperature, it seems difficult to explain why direct germination, which seems to be dependent upon free oxygen, as is shown by the present author's experiments, failed to occur when the cultures were placed in an oxygen-free atmosphere at 30–31° C. Of course it may be argued that, in this genus, the requirements of oxygen for the two types of germination are so different that, while the oxygen within the sporangium may stimulate one type of germination, it may be incapable of influencing the other without the aid of free oxygen in water. It is, however, for future research to show whether such is really the case.

In arriving at the third conclusion that, in *Phytophthora,* direct and indirect germination are two different phenomena, it may be questioned whether such a broad generalization seems warrantable from the experi-

mental data at hand. However, if we consider the material changes which occur in the process of spore germination in general, it will be realized that the process with which we are dealing in indirect germination of the conidia of the species of *Phytophthora* is hardly comparable to the one which occurs, for example, in the rusts. Considerable amounts of organic food materials are stored in all spores. During germination the germ tube utilizes the stored food material and continues its development as long as the reserve food in the spore is available. If, however, the culture medium does not contain any food material, the germ tube is hindered in its development and ultimately starved to death. Now if we may look upon the formation of zoospores as nothing but the rearrangement of cytoplasm followed by cleavage, it will seem reasonable to assume that the products of cleavage, viz., zoospores, should retain the food material necessary to sustain the growth of germ tubes. But if we may regard the liberation of zoospores as a process comparable to the production of germ tubes, it will follow that the process of indirect germination has deprived the conidium of its stored energy (in the form of reserve food material), and the zoospores are incapable of further development, just as a germ tube is, unless supplied with nourishment from the medium. But the zoospores, as we know, act as independent protoplasmic units endowed with the same power of producing a germ tube and sustaining its growth for a short time that is possessed by a conidium. Evidently, then, a zoospore is a physiologic equivalent of a conidium insofar as its ability to produce and sustain a germ tube is concerned. The process of the liberation of zoospores in *Phytophthora,* therefore, is not germination in the same sense as that process in a germinating rust spore; it is a process which prepares the conidium to multiply itself vegetatively, a fact very significant as an aid in the dispersal of the spores. A zoospore is, in fact, a sporangiospore, and the process which gives rise to it is the formation of sporangiospores.

It is very important to note that the process of indirect germination in *Phytophthora* is not the same as that in the other genera of the Peronosporales. In what ways a change in the physical influences of the surrounding medium will affect the complex material system within a sporangium of a species of *Phytophthora* and not that of the other genera of the Peronosporales is not yet easy to comprehend. It may be that the cleavage of the protoplasm in the sporangia of *Phytophthora* is more complete than in the other genera of the Peronosporales, so that the attempt on the part of a sporangium of this genus to throw out a part, or the whole, of its cytoplasm takes much less energy than is required in the other genera. It should also be mentioned in passing that it would be of great significance to examine some species of algae in order to determine whether the formation of zoospores in sporangia is influenced by the presence or absence of oxygen around the cultures.

SUMMARY

Conidia of *Phytophthora colocasiae* under proper temperature conditions were able to form zoospores in cultures from which the air had been exhausted by means of a vacuum pump. They also formed zoospores in cultures from which the oxygen was withdrawn by alkaline solutions of pyrogallic acid.

Indirect germination also occurred in the case of conidia of *Phytophthora infestans, P. palmivora,* and *P. parasitica* in the absence of oxygen.

Direct germination did not occur with the conidia of any of the species of *Phytophthora* used, when oxygen was excluded from the cultures.

The conidia of *Albugo candida, Plasmopara viticola,* and *Sclerospora graminicola,* which germinate indirectly, as well as the conidia of *Peronospora parasitica* and *Peronospora trifoliorum* which germinate directly, required the presence of oxygen for germination.

Direct and indirect germination are two different phenomena, the former being a type of growth, the latter a mere breaking up of the protoplasm into independent units.

IOWA AGRICULTURAL EXPERIMENT STATION,
AMES, IOWA.

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EXOBASIDIUM CAUCASICUM WORONICH. IN TRANSCAUCASIA AND KAMCHATKA

N. N. Woronichin

W. Tranzschel, in a personal conversation with me, expressed the supposition that the fungus, *Exobasidium caucasicum,* found on the leaves of *Rhododendron caucasicum* Pall. at the top of of the Zchra-Zkaro (Transcaucasia), and described by me (12), is possibly identical with the specimens of *Exobasidium* collected by the Academician, V. Komarov, in Kamchatka on the leaves of *Rhododendron chrysanthum* Pall.[1] and determined by W. Tranzschel as an *Exobasidium pentasporium* Shir. (11, p. 570). Therefore I again looked through the material of *Exobasidium caucasicum,* from Kamchatka, collected by the Academician V. Komarov, and the specimens of *Exobasidium pentasporium* Shir., collected on *Rhododendron rhombicum* Mig. in Nicco, determined by the author (from the herbarium of the Section of Mycology and Phytopathology, A. A. Jaczewski) and by others on the *Rhododendron sp.* from the collection of Samurai in the herbarium of the Botanical Museum of the Academy of Sciences, Tokyo.

I regret that it was impossible to study the structure of the basidia and spores (the latter, according to the description, are similar in dimension and shape to those of *Exobasidium caucasicum*) of the last two specimens because of the poor conservation of the hymenium of the parasite; but comparing the diagnostic description and drawings of Shirai (7, p. 53, Pl. IV, fig. 18–19), with the external aspect of the infection, it was possible to determine the undoubted difference between my *Exobasidium* and the fungus of Shirai, which may be seen from table 1.

Concerning the specimens from Kamchatka, they are identical with mine as to the exterior aspect of the infection and also as to the microscopical characters. I succeeded in observing but little difference as to the dimensions of basidia and basidiospores. The basidia of the specimens from Kamchatka measured 42–52 × 7–10 μ against 39.6–42.9 × 6.6–8.8 μ of the specimens from Transcaucasia, but the basidiospores of the specimens from Kamchatka measured 14–17.5 × 5–5.5 μ, so that they scarcely differed from those of the Caucasian forms, the dimensions of which are 14–16.5 × 5 μ. The number of spores on the basidia of the fungus from

[1] Specimen labeled "*Exobasidium pentasporium* Shir. on *Rhododendron chrysanthum* Pall. Kamtchatka, from Petroparlovsk to Kamtschakta Mt., near the vil. Ganal: the subalpine zone. June 18, 1909. Lg. V. Komarov."

Kamchatka was 4, while their shape is identical with that of *Exobasidium caucasicum*. It was often observed that the spores are septate and that they bud, producing fusiform conidia with little oil drops at the ends; the conidia are somewhat larger than those on the Caucasian specimens, reaching 7–10 × 1.75–3 μ, mostly 7 × 1.5 μ compared with 5 × 1.5 μ in the diagnosis of *Exobasidium caucasicum*.

TABLE 1.—*Comparison of Exobasidium caucasicum and Exobasidium pentasporium*

Structure	*Exobasidium caucasicum* Woronich.	*Exobasidium pentasporium* Shir
Hymenium	Constantly dispersed on the lower surface of the young leaves.	In most cases in patches, more seldom dispersed on the lower surface of the leaves of different age, sometimes leaving their margin free.
Basidiospores	4, seldom 2–3	4–6, commonly 5.
Branches	Does not form "witches' brooms"	Forms "witches' brooms"
Host plants	*Eurhododendron*	*Azalea* and *Tsusia*.

The discovery of my fungus on *Rhododendron chrysanthum* in Kamchatka, seems to me, clears up the question of the origin of the Caucasian *Rhododendron*.

Prof. N. Kusenezow presents the history of the genus *Rhododendron* in Caucasia as follows: "Concerning the other Ericaceae of the Flora Caucasica, they probably have developed in the Caucasus in the post-tertiary time and can be considered as . . . the further development of the tertiary types of Caucasia, e.g., *Rhododendron caucasicum, R. ungernii, R. smirnovii* and others, which changed after the tertiary time and became adapted to the new climatic environment in Caucasia after the ice period." (3, p. xi.) Such an ancient tertiary type of *Rhododendron* in Caucasus is *Rhododendron ponticum* L. according to Kusnezow, "an ancient type, near to the original tertiary form of the Caucasian *Rhododendron*, or, perhaps, identical with one of them. From such an ancient type, on the boundaries of Colchids and Armenia, probably came the endemical Caucasian Rhododendrons, *R. ungernii* Trautv. and *R. smirnovii* Trautv. From the same type, *R. caucasicum* Pall. arose in the mountains of Caucasus, and *R. chrysanthum* Pall. in the mountains of Siberia. The ancient type itself, *i.e., R. ponticum*, has remained in Colchid." (3, p. 13.)

The fact of the parasitism of *Exobasidium caucasicum* on *Rhododendron chrysanthum* on the one hand and its occurrence on one species of the Caucasian Rhododendrons, namely *R. caucasicum*, on the other hand does not

quite agree, I believe, with the aforementioned description of the genesis of the group of Rhododendrons in Caucasia.

In fact, excluding the possibility of transferring the parasite by atmospheric currents in the present, or even at a more ancient time, and recognizing our representative of the genus *Exobasidium* as an ancient type of fungus, it would be natural to find it parasitizing the primordial species of *Rhododendron* or the closely related species, *R. ponticum* L.

Such cases of parasitism of *Exobasidium caucasicum* on *R. ponticum* have not been observed up to this time, although this fungus is not rare in Transcaucasia and is often found, as evidenced by N. Troitzky's and my own observations.

I think that the facts of parasitism and the extreme resemblance between *R. caucasicum* and *R. chrysanthum* as shown by Maximowicz (4, p. 21); the similar ecology of the two species; and a somewhat taxonomic separatism of *R. caucasicum* from the other two species of this genus, known in Caucasus,[2] admit the endemism (or more exactly hemiendemism) of *R. caucasicum* as not equivalent to the endemism of the other two endemes of the genus *Rhododendron* in Caucasus. It seems to me possible to admit the migration into Caucasus of *R. chrysanthum* as such during one of the ice periods, perhaps together with the other fourteen emigrants from the high mountains of Asia as enumerated by Kusnezow (3, p. xxi). In its new habitat this species could be the progenitor of the contemporary *R. caucasicum* in the same manner as the arctic species of *Gentiana* have formed their separate races in Caucasus (3, p. xxvii).

The example, described by me, of the parasitism of *Exobasidium caucasicum* on two closely related, although geographically separated, species of *Rhododendron* recalls an analogous case, stated by Raciborski, which gave to this author the opportunity of expressing the following considerations concerning the particular occurrence of *Azalea pontica* L. (otherwise called *Rhododendron flavum* Don.) in Eurasia. This bush is known to grow in the Caucasus, in Asia Minor, in the North of Volhynia near Ovrutch, and in the year 1909 it was found also in the forest of Sandomir. In the latter country Raciborski has found in abundance on the leaves of *Azalea* the parent fungus, *Exobasidium discoideum* Ell., for the first time described in America on the related species of *Azalea viscosa* L. An almost identical form of this fungus, *Exobasidium discoideum* var. *horvathianum* Thom., has been found on the leaves of *Azalea pontica* L. in Caucasus.

[2] *Rhododendron ungernii* and *R. smirnovii* can be put between *R. ponticum* and *R. caucasicum*. Prof. Kusnezow, p. 22 of the publication cited, says: "The position of *Rhododendron ungernii* and *R. smirnovii* between *R. ponticum* and *R. caucasicum* as well as their geographical distribution give some reason for supposing that these two new forms are a hybrid of secondary generations."

Azalea pontica L., and *Azalea viscosa* L., correspond taxonomically with the representatives of the group "Azaleae genuinae," which grow in greatest abundance in North America and only a few of which ocur in the East of Asia. The relationship of the North American Azaleae and *Azalea pontica,* according to Raciborski, is demonstrated by the parasitism of the above mentioned *Exobasidium,* which undoubtedly developed on the primordial native Azaleae and has extended from there together with the different species of Azaleae in North American and Eurasiatic countries. Raciborski assumes that there could have been during the Myocene such a center of development of Azaleae in the extreme North, from whence the species extended to the South, in the direction of the meridians. It is interesting to remark that on the leaves of Azaleae in the forest of Sandomir, Raciborski found also the other form of *Exobasidium* whose independence from *Exobasidium discoideum* Ell. he doubted (5, p. 391). This fungus was insufficiently deserибed later by Raciborski in "Mycotheca polonica" under the name *Exobasidium dubium* Rac. (nomen nudum) (6, p. 1172).[3] At the same time this fungus was found by some other explorers in Anatolia and in Transcaucasia; a little later it was found in several countries of western Transcaucasia. In the Caucasian lists, the fungus was designated as *Exobasidium sp.,* and in the work of Bubák as *Exobasidium vaccinii* (Zûl.) Woronin. Forma. *Rhododendri flavi.* Bub. (1, p. 101). In the year 1913 the organism was thoroughly studied by me and named *Exobasidium magnusii* (13, p. 15). And so this species forms, as to its occurrence, a full parallelism with *Exobasidium discoideum* Ell., but apparently has not been observed in America up to this time.

Although these parallel but geographically separated forms of *Exobasidium* are fixed by me under three specific names, nevertheless it is possible that in each of these three species a more detailed study will establish morphological differences in accordance with the geographical distribution.

The measurements mentioned by me in the beginning of this treatise show in fact some unimportant differences in the dimensions of basidia and conidia, on the species of Caucasian and Kamchatkan *Exobasidium caucasicum.* The statement of Raciborski (5, p. 388) for the *Exobasidium dubium* differs a little as to the dimensions of basidia from the Caucasian *Exobasidium magnusii,* but, according to the information of Thomas (10, p. 305) the Caucasian specimen of *Exobasidium discoideum* differs from the American species by the still smaller dimensions of basidiospores which compelled the author to designate the Caucasian form under the name, var. *horvathianum.* I am sorry I could not find in the literature exact informa-

[3] Specimen labeled *Exobasidium dubium* Rac. (*Exobasidium* sp. Raciborski *Azalea pontica* 1909, p. 398, fig. 2.) Na lisciach *Azalea pontica* w Woli Zarzyckiej w puszczy Sandomierskiej. 20/6. 1909 Zb. M. Raciborski.

tion as to the dimensions of basidia and basidiospores of *Exobasidium discoideum* (5, p. 385, 8, p. 665, 9, p. 538). Burt (11, p. 645) gives the dimensions of the basidiospores only.

In general the question of the existence of morphological differences between the geographically isolated pairs of *Exobasidium* needs special study, but it is not improbable that in this case we have to deal with three pairs of elementary species, which differ in their distribution and in some unimportant morphological characters.

JACZEWSKI INSTITUTE OF MYCOLOGY AND PHYTOPATHOLOGY,
LENINGRAD, RUSSIA.

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TRANSMISSION OF POTATO SPINDLE-TUBER BY CUTTING KNIVES AND SEED PIECE CONTACT[1]

R. W. Goss

INTRODUCTION

The spindle-tuber disease repeatedly has been found to increase more rapidly in certain infected seed stocks in Nebraska than could be accounted for by the presence of known insect carriers in the field. While this increase in the disease possibly might be due to the presence of undetermined insect carriers, it was thought that seed piece contact and the cutting knife might serve as a means of transmission. The only reported attempts to secure infection in this way, with which the author is familiar, were made by Schultz and Folsom,[2] who report that they were unable to infect healthy tubers by means of the cutting knife in a limited number of tests. They also found that juice from infected tubers was not as virulent as juice from the green parts of the plant when leaf mutilation inoculations were made. The results would indicate that the infectious principle may not be in an actively virulent condition in the tuber, but we know, on the other hand, that infected tubers usually produce infected plants and that tuber grafts usually give a high percentage of infection. Tuber inoculations made by the author with plugs of infected tissue inserted into healthy seed pieces always have given a high percentage of infection. It appears then that the infectious principle present in the tuber is in a virulent state and capable of causing the infection of healthy seed pieces.

No attempt was made in this work to distinguish between spindle-tuber and unmottled curly dwarf as the symptoms of these two diseases have not been clearly differentiated on the Bliss Triumph variety. It is possible that unmottled curly dwarf as described by Schultz and Folsom was present in these tests.

A preliminary test was made in the greenhouse by cutting a healthy tuber into 4 seed pieces and inoculating 3 of them by rubbing the freshly cut surfaces with spindle-tuber seed pieces two or three times. The 4 seed

[1] Published with the approval of the Director as Paper No. 15, Journal Series, of the Nebraska Agricultural Experiment Station, Lincoln, Neb.

[2] Schultz, E. S. and D. Folsom. Infection and dissemination experiments with degeneration diseases of potatoes. Observations in 1923. Jour. Agr. Res. 30: 493–528. 1925.

pieces were planted in separate pots. All 3 inoculated seed pieces produced spindle-tuber plants, and the new tubers showed the characteristic symptoms of the disease (Plate XXI, A). The control seed piece produced a healthy plant with normal tubers.

EXPERIMENTAL METHODS

Further tests were made in the field in 1925, using both contact and knife inoculations. Healthy tubers were halved; one half was cut into 2 quarters with a knife disinfected in lysol, and these quarters were used for controls. An infected tuber was then cut in half with another knife, and this knife rubbed once across the freshly cut surface of the healthy half tuber and then used to cut it into 2 quarters. The procedure with the contact inoculations was similar, except that a sterile knife was used on each tuber and 2 of the quarters were rubbed once or twice with the freshly cut seed pieces of infected tubers. The inoculated seed piece, the healthy control from the same tuber, and the diseased control used as the source of inoculum were planted opposite each other in 3 rows, the healthy controls in the middle row. All the seed pieces were planted immediately after inoculation. The soil was moist, and conditions were ideal for sprouting. The plot was sprayed with Bordeaux mixture, containing both lead arsenate and nicotine sulphate, as often as necessary to keep out insects. Emergence records were taken and notes made every ten days. The progeny of each hill was harvested separately so that both yield and tuber symptoms of each inoculated plant could be compared with its healthy and diseased control plant. Fifty plants for each method of inoculation were grown in each row. The Bliss Triumph variety of potatoes was used in all the tests.

RESULTS

No differences were noted between the inoculated and healthy plants as to time of emergence; and the diseased controls sprouted almost as rapidly, probably owing to the ideal soil conditions at planting time.

The symptoms of the disease appeared on the spindle-tuber controls as soon as the plants were large enough to enable the symptoms to be distinguished. The inoculated plants which became infected had clearly observable symptoms of the disease a month after emergence.

Ten of the healthy control plants in each plot had symptoms of the disease both in the tops and the tubers; these plants, with the corresponding inoculated plants which were all infected, were discarded and not considered in the data from which table 1 was compiled.

As seen in table 1, infection was obtained by both methods of inoculation (Plate XXI, B and C), but a larger percentage of infection resulted

from the contact inoculation. All the plants listed as infected had the disease symptoms appearing on the tops, and the degree of severity of the symptoms on the tops corresponded with those on the tubers. The infected plants had a smaller total yield than the healthy controls, the average of both tests being only 52 per cent of the yield of the controls. There was also a smaller percentage of marketable potatoes from infected plants, both tests averaging only 42 per cent of the controls.

In addition to the infected plants listed in table 1, 19 plants, or 47.5 per cent of the inoculated plants in the knife inoculation test, and 18 plants, or 45 per cent of the contact inoculations, were questionable spindle-tuber. These plants showed some symptoms of the disease in the field, and the tubers were not entirely normal. The total yield per plant was only 83 per cent, and the yield of No. 1 tubers only 77 per cent of that of the healthy controls. The symptoms of the disease were not distinct enough to group them with the infected plants, although undoubtedly most of them would produce distinct spindle-tuber plants the following year.

There were thus only 8 plants, or 20 per cent of the plants inoculated with the cutting knife, which remained healthy on the same basis as the healthy controls, while only 3, or 7.5 per cent of the contact inoculations, remained healthy.

As stated above, 10 of the 50 controls showed typical spindle-tuber and were not considered in the above data. The corresponding inoculated plants also showed the disease, but in a more severe form; the yield was only half that of the infected controls, and the tuber symptoms were much more severe. It is possible that this may have been due to the additional inoculation of these infected seed pieces.

The contact inoculations not only gave a higher percentage of infection than the knife inoculations, but also resulted in more severe symptoms of the disease. The yield per hill was much less, and the symptoms on the tubers were more severe, as shown in table 1. This probably was not due to the method of inoculation, however, as the spindle-tuber controls also show the same differences. The half of the rows in which the contact inoculation set was grown received a little more irrigation water, and the soil moisture content was undoubtedly higher throughout the season. This is evidenced by the comparative yields of the healthy controls, which were 100 to 200 grams per hill higher in the well watered part of the plot. It is known that a high soil moisture content tends to increase the severity of the tuber symptoms of spindle-tuber, and the above differences probably can be accounted for on this basis.

DISCUSSION

The chief sources of error in an inoculation experiment of this type are reduced to a minimum in the experiments here reported. The possibility

TABLE 1.—*The results of inoculating healthy seed pieces with spindle-tuber by means of the cutting knife and by seed piece contact*

Treatment	No. of plants	Per cent of plants infected	Aver. total weight in grams of tubers per plant	Aver. wt. in grams of No. 1 tubers per plant	Total number of tubers	Tuber Symptoms[a]						
						H	?	1	2	3	4	5
						Per cent of total number of tubers						
Spindle-tuber controls	50	100.0	168	34	333	0	0	0	2.7	37.8	40.5	18.9
Healthy controls	40	0	1072	952	451	100	0	0	0	0	0	0
Inoculated by means of cutting knife[b]	40	32.5	814	694	122	28.7	4.9	26.2	22.1	11.4	6.5	0
Spindle-tuber controls	50	100.0	148	68	212	0	0	0	0	26.9	44.8	28.3
Healthy controls	40	0	1237	1085	497	100	0	0	0	0	0	0
Inoculated by seed piece contact[b]	40	47.5	391	175	199	3.0	6.0	3.0	24.6	31.1	19.6	12.6

[a] H = healthy; ? = questionable; 1 = very slight; 5 = very severe.

[b] All the data on weight, number, and symptoms of inoculated plants are based on the number of infected plants.

of the original seed tubers being infected is hardly plausible when the presence of disease in the inoculated row is between 30 and 50 per cent, with the controls from the same tubers remaining healthy. The possibility of current season infection would hardly account for the results, inasmuch as the control row was planted between the inoculated and diseased rows and all doubtful controls were discarded along with the plants from the corresponding inoculated seed pieces.

We must therefore conclude that spindle-tuber can be transmitted by cutting knives and by contact of freshly cut seed pieces. It is possible, also, that a picker planter could serve as a means of transmission. No evidence is at hand, however, to prove whether or not this actually occurs in practice, but the author can see no reason why the disease could not be spread in this way.

If this means of transmission is proved to be a factor in the spread of the disease, it will call for additional control methods. The sterilization of cutting knives is impractical, but the very rigid sorting out of all spindle-tuber seed before cutting would decrease this danger. It is possible, also, that healthy seed pieces which have come in contact with infected ones or with cutting knives used to cut infected tubers could be treated after cutting with a disinfectant and the control of the disease be increased in this way.

SUMMARY

Evidence is presented here which shows that the spindle-tuber disease of potatoes may be transmitted by cutting knives and by contact of freshly cut seed pieces. The results of the experiments may have an important bearing on methods of control.

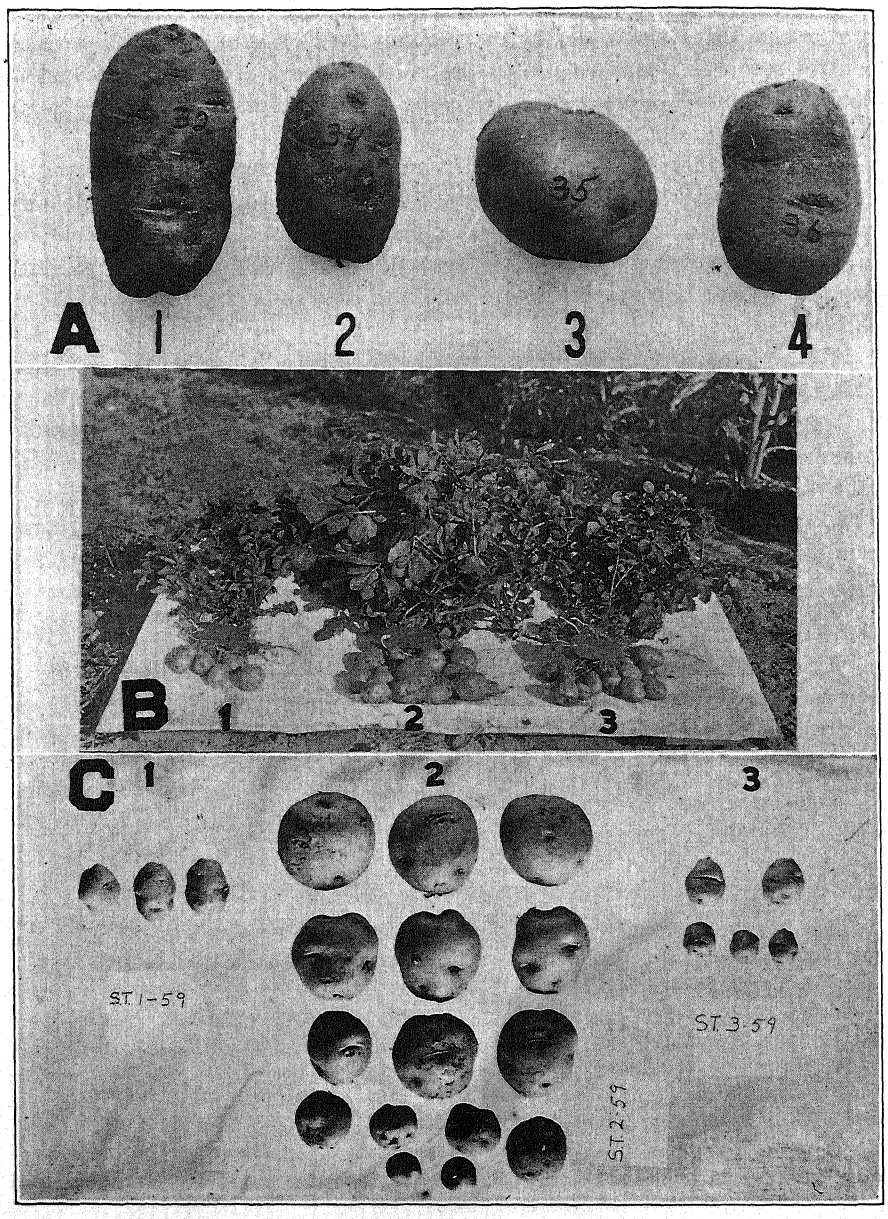
NEBRASKA AGRICULTURAL EXPERIMENT STATION,
LINCOLN, NEBRASKA.

EXPLANATION OF PLATE XXI

A.—Results of inoculations in the greenhouse made by rubbing together the freshly cut surfaces of spindle-tuber and healthy seed pieces. Tubers 1 and 2, progeny of 2 inoculated seed pieces. Tuber 3, progeny of healthy control from the same tuber as 1 and 2. Tuber 4, progeny of spindle-tuber seed used as the source of inoculum.

B.—Plants and tubers from cutting-knife inoculation test in the field. Plant 1, spindle-tuber control from seed used as source of inoculum; Plant 2, healthy control from same tuber as Plant 3, which was grown from a seed piece inoculated by cutting with a knife used to cut the seed piece for Plant 1.

C.—Progeny of seed-piece-contact inoculation test in the field. 1, progeny of spindle-tuber seed piece used as source of inoculum; 2, progeny of healthy control seed piece; 3, progeny of inoculated seed piece. The healthy control and the inoculated plant were from seed pieces from the same tuber.


A
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2
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THE FUSARIOSIS OF CEREAL CROPS IN EUROPEAN RUSSIA IN 1923

M. DOUNIN

FUSARIOSIS OF SMALL GRAINS

The summer of 1923 was extraordinarily rainy and it had many cloudy days. This probably accounts for the widespread prevalence of fungi of the genus *Fusarium* Lk. on the cereal crops of Russia. Numerous specimens obtained from different parts of the country give reason to believe that this fungus was disseminated in 1923 over a very considerable territory, the western boundary of which almost coincides with the western boundary of Russia. The western boundary is about the 52d meridian (from Greenwich); whereas the northern and southern boundaries extend from about the 62d to the 54th parallels, respectively.

In different localities of this region the percentage of infected grains varied considerably. It diminished southward and eastward. Most of the infected specimens were obtained from the region between Lake Ladoga and Lake Onega, and also eastward from the latter, covering the territory between the 32d and the 35th meridians east longitude and the 62d and 60th parallels north latitude.

In connection with the extraordinary prevalence of *Fusarium roseum* Lk. on rye, it was observed that the bread became poisonous (Inebriant bread). People who ate it suffered from weakness, vertigo, headache, nausea, and vomition. Table 1 shows the percentages of infected kernels in the specimens obtained from the region designated above. Besides the unusually high percentages of infected kernels, table 1 also points out that, in most of the infected grains, the fungus was found in a dead condition.

The above specimens were examined under the microscope immediately after the grain was harvested, and the infected kernels were then kept in a moist chamber for a period of from seven and one-half to eight months. If the cereals had been kept in the usual way so considerable a number of Fusaria (average 65 per cent) would not have died.

By studying the "biography" of each specimen we found out that the only way in which the Fusaria could be killed without damaging the grain was by drying the grain before threshing. The peasants of the above-mentioned region dry their grain in drying-sheds built especially for this purpose. These sheds are heated by stoves without chimneys. The sheaves

are put in the drying sheds and kept there for 12–18 hours at a temperature of from 46 to 54° C. The effect of the heat on the Fusaria is probably enhanced by such products of combustion as HCOH, SO_2, CO, etc., because of the absence of chimneys and because the door is kept tightly closed until the fuel is wholly burnt up.

Experiments were undertaken which showed that, after drying the grain in the described manner, a large percentage of the Fusaria were dead. It is possible that, under certain conditions, the drying-sheds may become a means of control against the fusariosis of cereals.

TABLE 1.—*The percentage of Fusarium-infection in grain crops in European Russia in 1923*

Specimen number	Infected crop	Percentage of infected kernels			
		Total	Percentage of kernels with viable Fusaria		Percentage of kernels with dead Fusaria
			Before chemical disinfection	After chemical disinfection	
1	Oats	17	1	0	16
2	Rye	92	30	0	62
4	Rye	56	12	1	44
5	Barley	90	7	3	83
6	Rye	91	1	2	90
7.	Oats	27	16	12	11
8	Rye	100	1	0	99
9	Rye	90	19	8	71
10	Oats	89	30	16	59
11	Oats	89	10	5	79
12	Rye	77	6	2	71
13	Rye	92	10	5	82
14	Rye	80	35	17	45
Average		73			65

An investigation of the degree of infection was undertaken. It has been shown that many, if not most of the kernels, are infected with Fusaria after harvesting when the sheaves are kept in shocks set up in the field.

The mycelium is found mostly in the superficial strata of the kernels, as oftentimes has been noted by many authors. A careful study of the character of mycelial distribution in the superficial strata of the kernels has shown that, in the specimens examined, the mycelium is not uniformly distributed. Most frequently it was found in the ends of the kernels, as is illustrated in table 2.

If we assume that the brush end of the kernel is not a good infection court during the ripening period and, in general, previous to harvesting,

TABLE 2.—*The percentage of Fusarium-infection in different parts of grain kernels*

Specimen number	Percentage of infection				Differences between		
	Entire kernel	Brush end of kernels	Middle portion of kernels	Germ end of kernels	3 and 4	3 and 5	5 and 4
			Rye				
2	92	92	66	90	26	2	24
4	56	56	44	44	12	12	0
6	91	91	82	91	9	0	9
8	100	100	89	66	11	34	−23
9	90	90	90	80	0	10	−10
12	77	37	44	77	−7	−40	33
13	92	92	92	92	0	0	0
14	80	80	70	70	10	10	0
Average	85	78.5	72	76	6.5	2.5	4
			Oats				
1	17	17	0	17	17	0	15
3	36	36	14	29	22	7	15
7	27	27	18	9	9	18	− 9
10	89	89	89	89	0	0	0
11	89	89	89	77	0	12	−12
Average	52	52	42	44	10	8	2
			Barley				
5	90	90	80	90	10	0	10

then we may consider the figures in column six as some indication of the way in which the Fusarium fungus penetrates the kernels after gathering.

INEBRIANT LINSEED-OIL

We also have obtained information concerning "inebriant linseed-oil" from the Gomel region. Specimens of flax seed from which the poisonous oil was obtained were found infected with a special form of *Fusarium*. A pure culture of this form was obtained, and, when compared with *Fusarium lini* Boll., it offered essential differences in the way of development of the

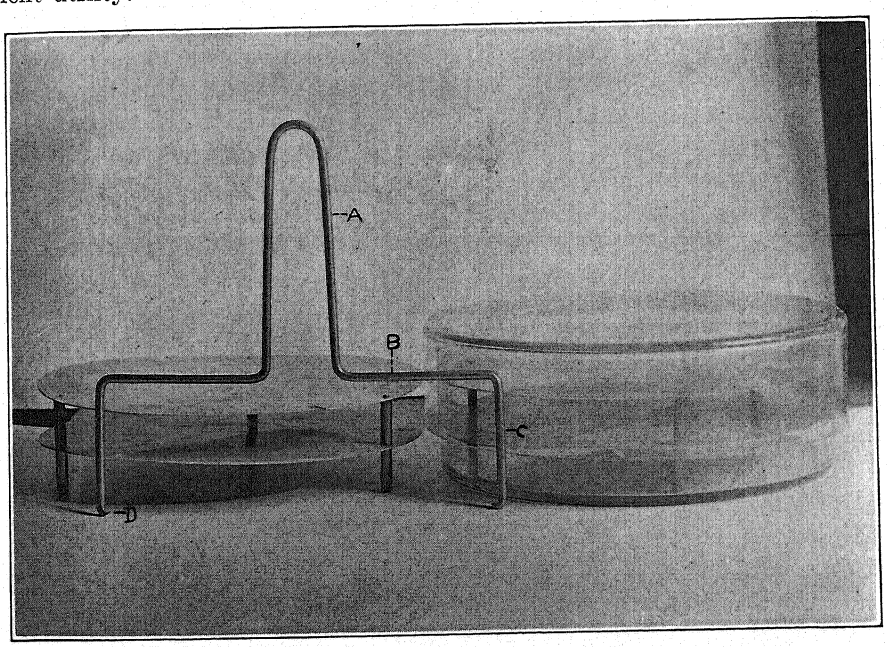
mycelium, in its color on nutritive media, and in the way of conidial formation.

As no other poisonous matter was found in these specimens of flax seed, and as the symptoms of poisoning by "inebriant linseed oil" are quite similar to those caused by "inebriant bread" (due to *Fusarium roseum*), this new form of *Fusarium* most probably is the cause of the inebriant characters of linseed oil which the population of certain regions of Ukraina use as a food product.

MOSCOW, RUSSIA.

PHYTOPATHOLOGICAL NOTES

Device for Increasing the Capacity of Moist Chambers.—The writer has found the following device useful in increasing the capacity of moist chambers where such are to contain culture slides or objects of somewhat similar bulk such as small Petri dishes. Essentially it consists of two zinc disks, one of which is supported from the bottom of the dish by three brass pins, and the second similarly upon the first one. The unit is removed from the moist-chamber or replaced by means of a lifter. While a similar equipment serving the purpose may be made more simply, there is added a description of this apparatus as used by the writer. It may be readily made from materials in the laboratory mechanician's shop, and is of permanent utility.



The dimensions given are those of the device fitting a glass moist chamber with a lid of 23 cm. inside diameter and with a tray of 21.5 cm. inside diameter. Two circular disks of zinc one half mm. in thickness are cut to a diameter of 21.2 cm. Slots are cut in each disk at opposite edges to a depth of 12 mm. and a width of 6.5 mm. It is important that these slots have the same relative position on each disk. Three holes, each 3 mm. in diameter, are drilled at points 6 mm. from the edge of the disk and 17 cm. apart. (Lines connecting these holes form an equilateral triangle.) It

is also important that these holes have the same relative position on each disk. Supporting and connecting pins for the zinc disks are made from brass rods 6 mm. in diameter. Three are cut to a length of 2.6 cm. and one end of each shouldered to a length of 5.5 mm., that part of the pin being threaded to fit in the small holes drilled in the disk, and to screw into the ends of the next set of pins. The latter three are 2.2 cm. in length, are drilled at one end to accommodate the pin set below them, and have a short shoulder at the other end to protrude through the small holes in the zinc disk resting upon them. These parts then are assembled as indicated in the accompanying figure.

The device is most conveniently removed from the dish for examination of materials by means of a lifter, made of flexible brass wire, or rod, 4 mm. in diameter, of the shape indicated in the illustration, and the free ends of which are obliquely faced off. Section A of the lifter has a length of about 12 cm.; B, 8 cm.; C, 6 cm.; while the oblique facing at D has a length of 3 mm. In using the lifter, its broad end is inserted in the slots of the zinc disks and the upper ends are compressed, which causes the lifter to engage the slots at their inner edge, when the arrangement may be brought out *in toto*. The top disk is then easily taken off, and the contents of both are available for examination.—N. M. GRIER, Dartmouth College, Hanover, N. H.

Occurrence of Club Root on Chinese Cabbage.—In a recent paper, W. H Davis[1] reported Chinese cabbage (*Brassica pe-tsai* Bailey) as a new host for the club root organism (*Plasmodiophora brassicae* Wor.). The disease was reported on this plant in 1912 by Cunningham[2] who considered it to be one of the seven most susceptible of the twenty-eight species studied.—F. L. WELLMAN, University of Wisconsin.

Results of Dutch Investigations.—In the forthcoming 32d volume of the "Tijdschrift over Plantenziekten" new results of Dutch investigations on diseases of potatoes, beans, cabbage, and other field and garden crops and some notes on forest pathology, will be published. The more important papers will contain summaries in English (sometimes in French or German).

Editors: Prof. Dr. Ritzema Bos, forester De Koning, Ir. v. Poeteren of the Plant Disease Survey, and Prof. Dr. Quanjer of the Institute for Phytopathology. Business manager: Dr. H. J. Calkoen, Dieren, Holland. Subscription price is $2.30 per year.—H. M. QUANJER.

[1] Davis, W. H. Club-root of Chinese cabbage. Mycologia 17: 160–162. 1925.

[2] Cunningham, G. C. The comparative susceptibility of cruciferous plants to *Plasmodiophora brassicae*. Phytopath. 2: 138–142. 1912.

PHYTOPATHOLOGY

VOLUME 16 NUMBER 5

MAY, 1926

STREAK OR WINTER BLIGHT OF TOMATO IN QUEBEC

T. C. VANTERPOOL[1]

WITH THREE FIGURES IN THE TEXT

An outbreak of streak, stripe, or winter blight of tomato, which was characterised by its sudden onset and virulent nature and which resulted in a loss of one-third of the crop, occurred on the tomato crop in the commercial greenhouses at Macdonald College in the spring of 1923.

Diseases of the tomato with apparently identical symptoms have been reported under a variety of names from widely separated parts of the world, and there seems to be considerable difference of opinion among investigators regarding their etiology. In view of this and the increasing importance of the greenhouse tomato industry in Quebec, a study of the disease under both field and greenhouse conditions was undertaken.

PREVIOUS INVESTIGATIONS

According to Orton and McKinney (16) the first mention of such a disease was by Lodeman in 1892, when he reported the occurrence of a blight of winter tomatoes in the greenhouses at Ithaca, N. Y., during the winter of 1890–91. In the same year Bailey (3) gave a fuller account of the trouble, to which he ascribed the name winter blight. Its primary cause remained undetermined, though the view was held that it was bacterial. Four years later Selby (19) described a blight of forced tomatoes in Ohio, with symptoms similar to those given by Bailey. Selby noticed that the youngest leaves showed earliest symptoms of the disease, all plants being liable to attack regardless of their thriftiness, and that most of the plants with diseased foliage had clean fruit. Winter blight of the tomato was reported from Pennsylvania in 1915, by Orton and McKinney (16), but the disease had been under investigation there for

[1] The writer is indebted to Dr. B. T. Dickson for many helpful suggestions, and for his kindness in affording field and greenhouse facilities.

some five years or more. They isolated in pure culture, light yellow and orange-brown, bacterial organisms from the lesions of diseased plants, but all attempts to produce infection by artificial inoculation were unsuccessful. Jackson (10) reported winter blight as having first appeared in the Indiana Station greenhouses in the winter of 1915. In 1916 the disease was first reported from Canada by Howitt and Stone (9). Repeated trials on their part to isolate a causal organism from the diseased tissues proved futile. These investigators are of the opinion that the disease is not seed transmitted, and carefully conducted experiments led them to believe that the cause of the trouble lies in the soil. In 1918–19, what appears to be the same trouble was reported from South Australia by Osborne (17), and

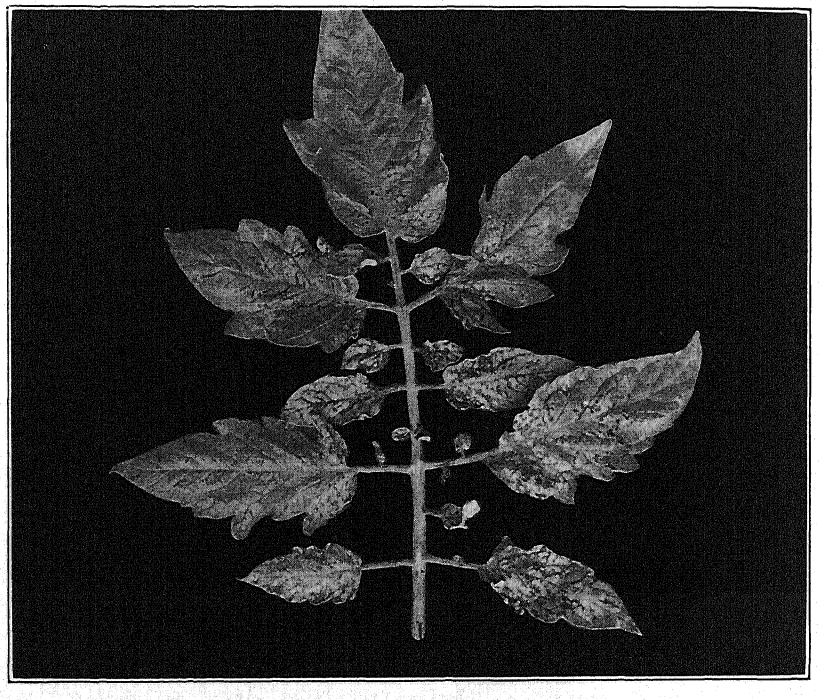


FIG. 1. A typical streak tomato leaf showing irregular necrotic spots.

from Victoria by Brittlebank (4). Two years later, 1921, Hamblin (8), from New South Wales, discussed a "spotted wilt" of tomato, which is probably none other than streak. Taylor (21) has reported it from New Zealand. In 1919 Paine and Bewley (18) published an account of a disease of tomato, in England and the Channel Islands, with symptoms apparently identical with those of streak or winter blight as described on this continent. These investigators isolated *Bacillus lathyri* Manns and

Taubenhaus, from diseased tissues, and with it they could bring about artificial infection of rapidly-growing tomato plants. Gardner and Kendrick (7), in a bulletin on tomato mosaic published in 1922, are of the opinion that the diseases described by Bailey of New York and Selby of Ohio, as well as those reported from Pennsylvania and Ontario, are mosaic in its most destructive form. Dr. B. T. Dickson, who reported the disease as being present in Quebec in 1923, inclined to the view that it is a mosaic in which necrosis occurs under certain environmental conditions. In a paper delivered before the Canadian Phytopathological Society in 1924, Stone (20) maintained that he could "produce the disease at will by using in excess fertilizers very rich in nitrogen, such as barnyard manure and ammonia or nitrates," and that the cause of the trouble is in the malnutrition of the plants.

From the foregoing historical sketch it is seen that three views are held regarding the etiology of streak or winter blight by workers on the problem, *viz:* (*a*) that the disease is caused by bacteria; (*b*) that it is of a purely non-parasitic nature, being brought about by malnutrition and improper management of environmental conditions in the greenhouse; (*c*) that the disease is a manifestation of mosaic in a most severe form.

SUMMARY OF REPORTS ON THE ECONOMIC IMPORTANCE OF TOMATO STREAK

Authority	Locality	Estimate of losses
Orton and McKinney (16)	Pennsylvania	5 per cent a fair average in greenhouses
Howitt and Stone (9)	Ontario	10 per cent in two greenhouses near Hamilton
Chupp (Correspondence)	New York	1 to 3 per cent in greenhouses
Thomas (do)	Ohio	Sporadic: 60 per cent in some greenhouses
Osmun (do)	Massachusetts	Some greenhouse crops almost ruined
Dickson (Observation)	Quebec	1923. One third of the greenhouse crop
Poole	New Jersey	Streak and filiform conditions caused 95 per cent loss in several greenhouses. (Plant Dis. Reporter Suppl. 25: 61. 1923.)
The writer's observations	Quebec	1924. 2 per cent in one greenhouse crop

GEOGRAPHICAL DISTRIBUTION

In North America the disease is probably most serious in Pennsylvania, New York, New Jersey, Northeastern Ohio, and Southern Ontario; and as reported to "The Plant Disease Survey" and according to Orton (15), it is also found in Indiana, Massachusetts, Michigan, Kansas, California and Washington. It has been observed in greenhouses in Maryland.

In appears to be more prevalent in the greenhouse than in the field, and on the autumn tomato crop than on the spring crop.

SYMPTOMS

An attempt to describe a set of clear-cut symptoms of the streak disease of tomato would necessarily be inadequate because of the various forms which the disease takes, depending apparently upon the succulence and age of the plant, as well as upon the temperature, illumination and other environmental factors.

In Quebec the disease usually is first noticed on tomato plants about the same time that the first blossom truss appears. Necrotic streaks on stems and spots on leaves occur in a zone about a foot in extent two-thirds up the plant. In this region irregular yellow spots appear on the leaves and subsequently turn brown and finally quite dark, being surrounded by yellowish areas (Fig. 1). Complete wilting of the leaves in this zone often results. Should optimum conditions for the disease continue to prevail, complete necrosis with wilt and drying out will occur. If, on the other hand, a slower growth is induced, or if the plants are hardened off slightly, then the upper growth becomes spindling and distorted; the leaves small and ruffled, weak, crinkled and curled downwards with numerous yellowed and minute necrotic areas. At this stage these plants invariably show marked symptoms of mosaic as well. On looking down the rows between the greenhouse beds, the spindling growth of the upper part of the plant is very prominent and once seen is unmistakable thereafter. Infected plants usually linger on in this condition for several weeks, and may even produce a moderate crop of fruit, only part of which will be blemished by spots; but even in this condition these plants serve as sources of infection for healthy plants.

Immediately below the diseased zone there are on the average 8 to 10 strong, apparently healthy, mature lower leaves. These seldom have lesions, but the young shoots which arise from the axils of these leaves usually have typical symptoms.

The brown linear lesions on the stem vary from one millimeter to several centimeters in length (Fig. 2). They enlarge and often coalesce, although this is a variable condition depending on the duration of optimum conditions for the disease. The lesions on the petiole are often so large that

they cause it to shrivel, turn brown and dry out with the leaflets hanging dead and dry.

The lesions on the flower-stalk and sepals are small and not very elongated. Similar spots occur on petals and stamens.

All the fruit on a diseased plant may be entirely free from outward blemishes or a portion of them may show lesions. In the same truss some fruits may be spotted and others apparently healthy; also, some trusses of fruit may show signs of the disease while others on the same plant may

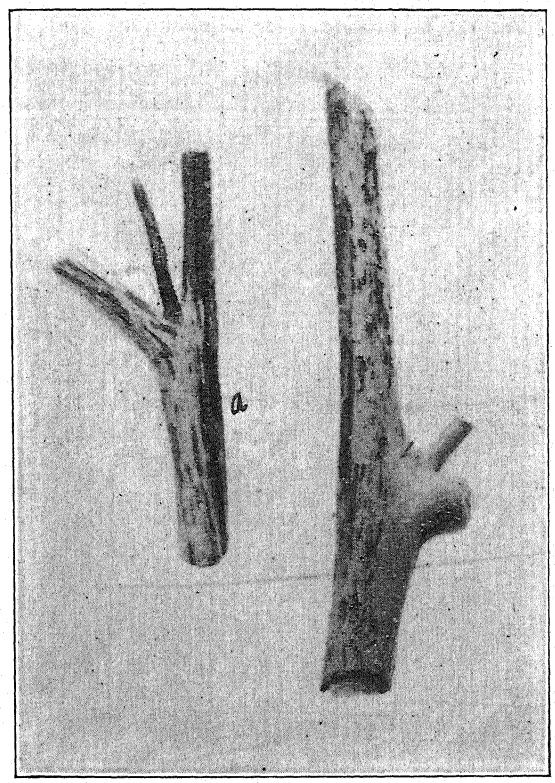


FIG. 2. Brown, slightly sunken, streak lesions on stems of tomato. Coalescence is seen at "a."

be normal. Plants have been found with much of their fruit diseased but typical streak lesions on stems and leaves were entirely absent; these plants, however, had definite symptoms of mosaic in varying degrees of severity. Symptoms on the fruit differ according to the variety of tomato. For the most part, irregular, raised, brown, greasy patches with no definite arrangement occur on the fruit (Fig. 3.) These frequently coalesce and form

quite large sunken patches in which cracks or slits soon develop owing to their drying out and the subsequent growth of the fruit. Severely affected fruits often are deformed.

An examination of the roots, both externally and internally, reveals no apparent divergence from the roots of normal plants, except that perhaps the root system is reduced somewhat.

At Macdonald College, plants affected with streak undoubtedly had mosaic. In many cases the mosaic symptoms were not clearly apparent at first; but, providing the plants did not die forthwith from the streak, the characteristic symptoms of mosaic always developed later, and usually in a most severe form.

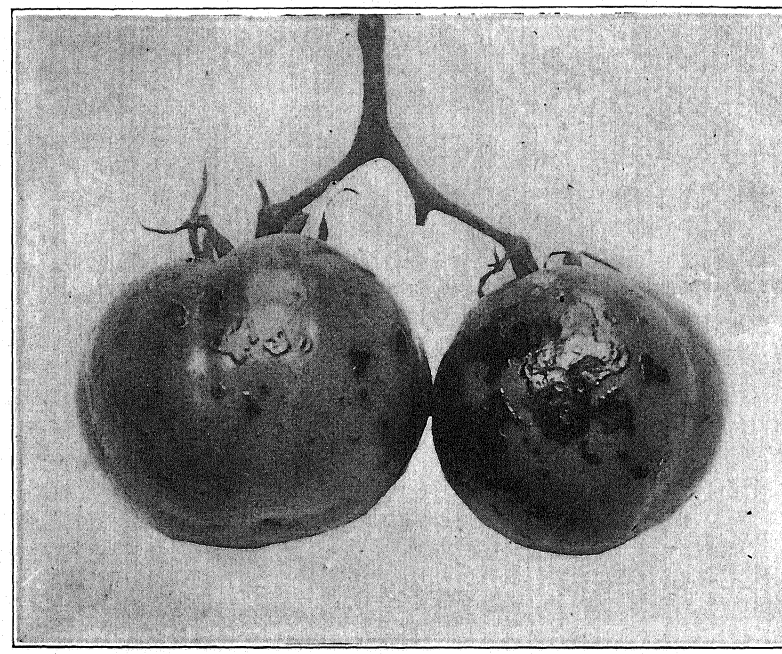


FIG. 3. Raised, brown, greasy patches on fruit of Livingstone Globe tomato. The older lesions are sunken and cracked because of desiccation of the cells beneath.

Internal symptoms.—Cross sections of diseased stems show that usually the lesions are merely superficial, but in some the necrosis extends well into the cortex, and in others the pith is invaded as well. The vascular elements do not show any definite necrosis.

All the tissues of the leaf, except the vascular bundles, become necrotic. There is a shrivelling and drying up of the lamina in affected areas.

The fruit spots involve only a few layers of cells, but occasionally minute patches of diseased tissue are noticed here and there within the fruit.

EXPERIMENTAL STUDIES

The experimental investigations upon which this paper is based were conducted under field conditions during the summers of 1924 and 1925, and in the hothouse during the intervening winter. Space did not permit of dealing with large numbers of plants and, to overcome this difficulty, the experiments were carried out on a small number of plants on three, four, or more, separate occasions. Further, by conducting the experiments both in the greenhouse and in the field, results of comparative value from an environmental standpoint have been added. Therefore the writer thinks that the paucity in the number of plants was overcome, and that the results are actualities.

All field inoculations were made just before dusk and on rainy evenings whenever possible. If no rain had fallen, the plants were thoroughly drenched with water before inoculation. Inoculations in the greenhouse were performed at any time during the day.

The soil in the experimental field plots was a loam which had received a dressing of farmyard manure the previous autumn; that used in pots in the greenhouse was a rich sod soil, to which farmyard manure was added.

In all experiments conducted in the greenhouse care was given to the control of aphids and white flies.

Bacterial Isolations and Inoculations

Numerous attempts to isolate a causal organism from diseased tissue were made. Most of these yielded no growth whatever, but a small percentage yielded a yellowish bacterial growth, which on plating out gave numerous yellow colonies. What appeared to be a similar organism was isolated from superficial and internal lesions both from the fruit and from the stem. Only occasionally could it be obtained from diseased leaves. This yellow organism is so irregularly found, however, that it can hardly be said to be constantly associated with streak.

Fresh bouillon cultures of the various strains of the yellow organism obtained from fruit, stem, and leaf respectively, of diseased plants both from the field and under glass, were repeatedly sprayed on young healthy tomato plants, some of which were wounded beforehand by rubbing the leaves gently between the finger and thumb. Similarly, cultures on nutrient agar were pricked into the stems of other plants and the wound covered with tin foil. After all such inoculations the plants were covered with large bell jars for two or three days. Sometimes a piece of sterile

cotton which had been soaked in a nutrient solution of the organism was wrapped around the needle-pricked host part and then covered with tin foil. All precautions to keep the plants from drying out were observed. On no single occasion under controlled conditions did streak develop as a result.

Through the kindness of Dr. Sydney G. Paine, Imperial College of Science and Technology, London, the author obtained a culture of *Bacillus lathyri,* which was isolated from clover, but which is identical in its physiological behavior with the strain from tomato, according to Dr. Paine. All artificial inoculations with this organism into rapidly-growing, healthy tomato plants failed to produce typical streak symptoms after a month and a half. This experiment was tried repeatedly. The fact that this organism had been growing in culture for nearly three months when the inoculations were made possibly might account for its loss in virulence.

A series of preliminary comparative cultural studies of strains of the yellow organism isolated from streak tomato plants with the strain of *Bacillus lathyri* obtained from Dr. Paine were carried out. These tests point strongly to the fact that the organisms are identical, although further tests will have to be carried out before such can be stated definitely. In all the reactions observed the results secured with the different strains showed no more variation than is common among strains of the same species.

Preliminary Experiments

A preliminary series of inoculation experiments in which both tissue and juice of streak tomato plants were inoculated into healthy tomato plants revealed the fact that infection may be brought about both by rubbing the leaves with diseased tissue and by inserting it into the stem, or in any way in which the extracted juice of diseased plants is brought into contact with that of the healthy plant. Thereafter all artificial inoculations were made either by rubbing the leaves of healthy plants with a small amount of crushed and macerated tissue or with the expressed juice of streak plants, or by pricking in such material with a sterile needle after first placing it on the plant.

Of 57 plants in these early studies so inoculated, over 85 per cent developed streak and mosaic, 8 to 10 per cent contracted mosaic only, and 1 to 2 per cent showed no disease symptoms. Only very occasionally was it difficult to decide to which group a plant belonged. Plants of all ages, and consequently of varying degrees of thriftiness, were thus experimented with; but the juices from streak plants were of such a virile and infectious nature that the disease was almost invariably produced, although the incubation period varied with the plant. In the field, however, on young plants growing under ordinary summer conditions, the incubation period

was invariably 12 days. Under greenhouse conditions with varying light no such regularity obtained during the winter months; but here by far the greater number of plants showed their first lesions in from 11 to 18 days. All control plants, inoculated with macerated tissue from healthy plants, remained healthy.

Systemic Nature of the Disease

As has already been mentioned under symptoms of the disease, there are many mature leaves on the lower portion of diseased plants, which from macroscopic examinations appear to be absolutely free from all malformations, lesions or discolorations of any sort. Several such leaves were carefully removed from diseased plants, and after immersing them in mercuric chloride (1:1000) for one to two minutes, they were washed with sterile water and crushed in a mortar. Healthy tomato plants were then inoculated with this pulp in the usual way. This experiment was repeated four times and in each case streak developed as a result.

An analogous experiment was conducted with clean, unspotted fruit, apparently free from any symptoms of the disease whatsoever, found on otherwise diseased plants. Such fruit also proved to be infectious.

It may be gathered from these experiments that while there are certain mature leaves and fruit which appear perfectly normal, they still serve as carriers of infection for the developing shoots of the same plant and as sources of inoculum for healthy plants. It shows further that streak is a disease which is visible in comparatively nascent tissue only, though all parts of the plant above ground carry the infective principle.

Whether the roots of streak plants are also carriers of infection still requires to be determined.

Nature and Properties of Streak Tomato Juice

The effect of filtration on streak juice.—Expressed juices from leaves, stems and fruit of streak tomato plants were passed through sterile Chamberland F filters into sterile flasks. Under aseptic conditions this filtered juice was put into sterile test tubes from which it could be used at once as a source of inoculum, or kept until required.

Stained preparations of this filtered juice were examined carefully for any microorganisms. As a further check on the freedom of the filtered juice from bacteria, nutrient agar slopes and nutrient broth tubes were also inoculated in duplicate, and incubated at 22° to 26°C. No growth resulted from such filtered juice.

A portion of the unfiltered juice was always saved and inoculated into healthy tomato plants so as to serve as a check on the viability of the streak juice before passage through the filter. The results of the inoculations are given in table 1.

TABLE 1.—*Results of inoculating healthy tomato plants with expressed streak tomato juice which had passed through a Chamberland F filter*

No. of tomato plants inoculated	Inoculum	Results		
		Streak	Mosaic only	No positive results
Greenhouse 1924				
11	Filtered streak juice (bacteria free), plants wounded	7	4	0
1	Filtered juice sprayed on plant (without wounding)	0	0	1
1	Filtered juice poured on soil	0	0	1
Field 1924 and 1925				
11	Filtered streak juice (bacteria free), plants wounded	11	0	0

In every case where streak developed the incubation period varied from 11 to 15 days. The results show conclusively that streak of tomato in Quebec belongs to the filterable-virus group of plant diseases.

The effect of high temperature on the power of infection of streak juice.—This experiment was carried out with a view to ascertaining whether the thermal death point of the infective agent lay in the range of the thermal death point of bacterial plant pathogenes or in that of the filterable viruses.

Ten cubic centimeters of the expressed juices from diseased plants were placed in test tubes with a pipette, care being taken not to allow any juice

TABLE 2.—*The effect of heat on the infectivity of the expressed juice from streak tomatoes*

No. of tomato plants inoculated	Treatment of streak juice used for inoculation	Results		
		Streak	Mosaic only	No positive results
1	45° C. for 10 min.	1 (11 days)	0	0
1	55° C. do	1 (15 days)	0	0
1	65° C. do	1 (13 days)	0	0
5	70° C. do	3 (12–14 days)	1	1
1	75° C. do	1 (15 days)	0	0
2	80° C. do	1 (13 days)	0	1
2	90° C. do	0	0	2

to get on to the sides of the tubes. These tubes were then placed in water held at various temperatures for 10 minutes and cooled under the tap immediately on removal. Inoculations were then performed in the usual way.

To serve as a check on the infective nature of the heated juices, plants were inoculated with unheated juice at the same time that the inoculations with the treated juices were made.

The results of these experiments show that expressed streak tomato juices heated 20° C., or more, above the thermal death point of the majority of bacterial plant pathogenes (*Bacillus lathyri* T.D.P. 50° C.) still retain their infective powers. Also, the exact temperature to which they may be heated without losing this power seems to vary with the source of the juice. This peculiar characteristic has been demonstrated by Allard (1) to be a property of tobacco mosaic, the thermal death point of which he found to vary from 80° to 90° C. for 10 minutes exposure. Doolittle (6) has shown that for cucumber mosaic the thermal death point is about 70° C.

The infective agent for tomato streak, therefore, has a thermal death point lying within the same range as that of other so-called virus diseases.

The effect of drying on streak tomato tissue.—Portions of diseased plants were air-dried at room temperature until thoroughly dry. This material was then ground to a fine powder by passing through a meat chopper, and stored in glass-stoppered bottles until required. For inoculation purposes a water-extract was prepared by soaking some of the fine powder in sterile water over-night. Diseased material which had been stored for two, three, and nine months was used. In no single case did streak develop, but definite mosaic, usually of a very severe nature, invariably was produced instead.

TABLE 3.—*Results of inoculating healthy tomato plants with streak juices after treatment with various strengths of mercuric chloride solution*

No. of plants inoculated	Inoculum		Length of treatment in hours	
	Treated streak juice (Gms. $HgCl_2$: c.c. streak juice)			
1	1	500	2.5	Healthy
1	1	500	24	do
1	1	500	48	do
1	1	800	2.5	Streak (15 days)
1	1	800	24	Healthy
1	1	800	48	do
1	1	1000	48	Streak (15 days)
1	Untreated streak juice (check)			Streak (12 days)

The effect of various chemicals on streak juice.—Mercuric chloride dissolved in water was added to streak tomato juice so that the resulting solution would be of the required dilution. Five cc. portions were drawn off from time to time for experimental purposes as required, and at time of inoculation this treated juice was diluted with an equal amount of sterile water so as to prevent injury to the tomato plants from the mercuric chloride itself. The inoculations were made by crushing the leaf with the finger and thumb and rubbing in the inoculum. The results are given in table 3.

One cc. of the inoculum was plated out in each case at time of inoculation but no growth whatever appeared on the plates at any time. From the above it appears that the streak juice can withstand mercuric chloride in concentrations fatal to bacteria.

Effect of Various Fertilizers in Relation to the Disease

From observations made on outbreaks in the college greenhouses, there appeared to be some connection between the vegetative growth of the plant and the incidence of the disease. Paine and Bewley (18) found that the application of potash largely counteracted the susceptibility of tomato plants to the stripe disease which they investigated; and Stone (20) recommends in his control suggestions that both acid phosphate and potassium sulphate be added.

TABLE 4.—*The influence of fertilizers on the incidence of the disease*

No. of tomato plants inoculated	Inoculum	Fertilizer treatment	No. of days after which streak developed
Field 1924			
5	Virile juices of streak tomato plants in all cases	No treatment	12[a]
5		Farmyard manure—heavy application	12
5		Acid phosphate, 3 oz. per plant	12
5		Sodium nitrate, 1 oz. per plant	12
5		Potassium sulphate ¼ oz. per plant	12
5		No treatment	12
Greenhouse			
2		Sodium nitrate	12 each
1		Manure + nitrate	14
2		Potassium sulphate	12 and 13 respectively
2		Acid phosphate	13 and 15
1		Acid phosphate + potassium sulphate	12

[a] One plant developed severe mosaic only.

Accordingly one series of manurial treatments was conducted in the field in 1924 and another in pots in the greenhouse during the winter. The results of both series of experiments are given in table 4.

In the field the fertilizer was applied one week before inoculation. In the greenhouse experiment the fertilizer applications were at intervals of one week beginning when the tomato seedlings were four inches high. The amount applied was not measured in this case, but the doses were very heavy. The inoculations were made between the second and third applications.

It may be gathered from these results that soil treatments alone cannot prevent infection when the plants are artificially inoculated with viable streak tomato juices. The disease appears after the usual incubation period regardless of fertilizer treatment, but the severity of the disease is slightly more pronounced on plants with the most succulent growth. Providing other environmental conditions are favorable for the development of streak, the plants will not completely "grow out of" the disease, even if the various fertilizer treatments are continued. The addition of chemical fertilizers alone, threfore, will not control streak; but, insofar as it prevents over-succulence in growth, it will help to ward off the disease, especially if other environmental conditions are carefully attended to.

Seed Transmission

Since tomato fruits are sometimes very severely affected, though for the most part only superficially, it might be expected that the disease possibly is transmitted by the seed. Orton (15) lists tomato streak among the seed-borne diseases, and attributes its recent appearance in widely separated parts of the world to this fact.

On three different occasions seed from severely spotted fruit was removed from the pulp, dried and then sown. As streak failed to develop in any of the plants from such seed, it must be inferred that tomato streak is not usually seed transmitted. The fact that necrotic areas within the fruit often line the ovary wall, however, suggests the possibility of occasional seed transmission.

Insect Transmission

Transmission of streak by aphids was readily demonstrated under controlled conditions by allowing aphids, ordinarily found on tomato, to suck the juices of streak tomato plants for a few days and then placing them carefully with a camel-hair brush on vigorously-growing healthy tomato plants. In over 75 per cent of the plants streak symptoms appeared after the usual incubation period.

Three attempts to ascertain whether white flies transmitted the disease were made, but in every case the plants remained healthy.

Developmental Relations of Streak

Temperature relations.—Healthy plants inoculated with streak tomato juice and placed in a hothouse maintained at a temperature of 65° to 75° F. during the day, developed symptoms of streak in 11 to 14 days as a rule. But it was noticed that similarly inoculated plants in the house maintained at 55° to 65° F. took a longer time for symptoms to appear. In several such cases where symptoms were not evident in three weeks, the plants were removed to the warmer greenhouses and in one to three days typical streak lesions appeared.

These results are only indicative since it was difficult to keep the greenhouse at a constant temperature from day to night; but they show, nevertheless, that higher greenhouse temperatures are more favorable to the devlopment of streak than the lower temperatures. Carefully controlled temperature-chamber experiments would probably produce interesting results.

On the other hand, if streak tomato plants growing under conditions suitable for streak development are hardened off by subjecting them to lower temperatures, the new shoots which appear subsequently will show little or no indications of streak.

An endeavor to induce the disease by manipulation of environmental factors.—Several series of rapidly-growing tomato seedlings were placed in soil consisting of one part of well-rotted manure and three parts of sod soil. Both healthy seedlings and young mosaic plants were included in the experiment. Small amounts of sodium nitrate were added to some of these plants about every seven days; to others, ammonium sulphate was applied. All the plants were supplied abundantly with water and kept at the higher greenhouse temperatures which were known to be favorable to the development of streak.

All attempts to induce streak in these plants by forcing their growth with nitrogenous fertilizers, by avoiding the use of acid phosphate and potash, and by over-watering and then allowing to become dry, produced negative results. Contrary to this, Dr. Stone, in a paper presented before the Canadian Phytopathological Society at Ottawa in December, 1924, maintains that he can produce the disease at will by using excess nitrogenous fertilizers, but in no case does he mention mosaic symptoms on these plants.

Johnson (11 and 12) and Tomkins (22) have shown that if mosaic potato or tobacco plants are subjected to temperatures unfavorable to the development of mosaic, then the symptoms will disappear; but when these plants are placed under optimum temperatures for their respective type of mosaic, the mosaic symptoms will return. From the study of the temperature relations elsewhere outlined, the same appears to be true for tomato streak. It is possible, therefore, for tomato plants to be growing

under conditions which would mask all streak symptoms and yet themselves be carriers of the agent of streak. Atanasoff (2) has shown that there are varieties of potatoes which serve as carriers of potato streak, but which do not themselves show any symptoms.

Cross Inoculations

Tobacco. Preliminary experiments showed that when healthy Resistant White Burley tobacco plants were inoculated with macerated tissue or extracted juice of streak tomato, either by rubbing the leaves or by pricking the plant through the applied juice, a mosaic of a very serious nature developed almost without failure in 10–14 days. Filtration through Chamberland F filters did not destroy the infectivity of this juice. The expressed juices from these infected tobacco plants were inoculated into healthy tomato plants both in the field and in the greenhouse. Table 5 contains the summarized results.

TABLE 5.—*Results of inoculating healthy tomato plants with juice of mosaic tobacco developed from tomato streak*

No. plants inoculated	Host	Source of inoculum	Streak	Mosaic only	No positive results
16	Greenhouse tomato plants	Juice of mosaic tobacco developed from streak tomato	11	3	2
9	Field tomato plants	Do	6	3	0
2	Greenhouse tomato plants	Do Juice passed through a Chamberland F filter	1	0	1

From such inoculations typical streak, followed later by mosaic, was produced in 69 per cent of the tomato plants. Clinton (5, p. 373) describes a bacterial-like burn produced in tomato from mosaic or calicoed tobacco. From Plate XXIX in this reference in which leaves and stems of this tomato plant having burn are shown, it would appear that this so-called burn is identical with streak or winter blight.

Other hosts. The results of similar cross inoculations, using ground cherry (*Physalis heterophylla*), pepper (*Capsicum annuum*), and eggplant (*Solanum melongena*), as intermediate hosts, indicate strongly that these Solanaceous plants may sometimes act as carriers of streak in a way similar to tobacco in the experiment outlined above. Further work on this phase of the problem is necessary.

Effect of Inoculating Tomato with Combination Viruses

The fact that mosaic is invariably present on streak tomato plants, and that streak material which has been dried for months produces mosaic only, when inoculated into tomato, suggested that there might be some other factor, possibly another infective agent which, together with tomato mosaic, produced the disease known as streak. This other factor presumably could not withstand prolonged drying. To show that true streak was not a severe manifestation of tomato mosaic only, the author subjected tomato plants showing pronounced mosaic symptoms to those conditions which had been shown to favor the development of streak most, and in no single case did true streak symptoms appear.

The publication of Johnson's findings (13) substantiated this view. This investigator, by inoculating a combination of potato mosaic and tobacco mosaic virus into tobacco and tomato, obtained leaf spots and blotches which sometimes killed entire leaves. This suggested the possibility that the other factor for which the present author had been searching was potato mosaic.

Tomato mosaic and potato mosaic combined. Juices were expressed from potato plants infested with mosaic and from mosaic tomato plants. Healthy, vigorously-growing tomato plants were inoculated, some with potato mosaic virus, some with tomato mosaic, and others again with a mixture of these two juices. The complete series which has been tested twice in the field and twice in the greenhouse is summarized in table 6.

The symptoms obtained when tomato mosaic and potato mosaic viruses were used were identical with those of tomato streak produced under similar conditions. Such symptoms did not develop in plants inoculated with tomato mosaic alone or potato mosaic alone. This streak derived from the combination virus could thereafter be transmitted to tomato plants.

These results at once opened up a new field of study. Since tomato and tobacco mosaic were known to be interchangeable, the latter was mixed with potato mosaic and the combination virus inoculated into tomato, with similar results as shown in table 6, exp. 3.

It also suggested the possibility of obtaining streak by inoculating mosaic tomato plants with potato mosaic, or, conversely, inoculating tomato mosaic into tomato plants which had three weeks previously been inoculated with potato mosaic. Accordingly, one of the plants of inoculation exp. 4, and one of exp. 5, table 6, were inoculated with potato mosaic three weeks after their first inoculation. At this time both plants showed definite mosaic symptoms only. Similarly, three weeks after the first inoculation, one of the plants of table 6, exp. 6, was inoculated with tomato mosaic. In all of these cases streak symptoms invariably developed 10–15 days after the second inoculation (table 6, exp. 8 and 9).

Further evidence that streak was caused by the combined viruses of tomato and potato mosaic was adduced when streak was obtained in mosaic tomato plants on which aphids from mosaic potato plants had been placed two to three weeks previously.

TABLE 6.—*Effect of inoculating healthy tomato plants with a combination of potato and tomato viruses*

Experiment No.	No. of tomato plants inoculated	Inoculum	Results 11–15 days after inoculation[a]
1	2	Tomato streak	Mosaic + streak
2	4	Tomato mosaic + potato mosaic	Mosaic + streak
3	4	Tobacco mosaic + potato mosaic	Mosaic + streak
4	2		Mosaic
5	2	Tobacco mosaic	Mosaic
6	2	Potato mosaic	No positive results
7	2	Control plants	Healthy throughout
8	2 mosaic plants	Potato mosaic	Mosaic + streak
9	2 plants inoculated 3 weeks previously with potato mosaic	Tomato or tobacco mosaic	Mosaic + streak

[a] Observations 4 weeks after inoculation showed no change.

Dried tomato streak material combined with potato mosaic. When streak tomato tissue, which was highly infectious when fresh, was dried for several months, then macerated and inoculated into tomato plants, mosaic only was produced. Potato mosaic virus was mixed with some of the macerated material and then inoculated into healthy tomato plants. This experiment was conducted both in the field and in the greenhouse with the results shown in table 7.

The symptoms which developed in all of the plants in table 7, exp. 2, were apparently identical with those produced on tomato plants with the fresh streak tomato material several months previously. From the above results it would appear, therefore, that the potato mosaic virus is the "factor" in the streak tomato material which was destroyed by drying.

Potato streak combined with tomato mosaic. In some recent preliminary experiments to ascertain if any relationship existed between potato

TABLE 7.—*Results of inoculating healthy tomato plants with macerated dried streak tomato tissue combined with potato mosaic*

Experiment Number	Number of tomato plants inoculated	Inoculum	Results
1	4	Macerated dried streak tomato tissue	Severe mosaic only produced
2	4	Do + potato mosaic	Mosaic + spots on leaves and streaks on stem (streak)
3	2	Potato mosaic	A peculiar mottling, distinct from tomato mosaic, was evident in some cases

and tomato streak, 8 tomato plants were inoculated with potato streak (from Spaulding Rose variety obtained from the Province of New Brunswick) and 8 with the combined viruses of potato streak and tomato mosaic. In the former case 5 of the plants produced a peculiar mottling distinct from true tomato mosaic, and 3 showed no apparent changes, whereas in the latter case, streak developed in all the plants. All controls remained healthy. Although only a few preliminary experiments have been attempted, the writer has been unable to produce streak in tomato from potato streak alone. The relationship between streak of potato and streak of tomato needs further elucidation.

A single series of inoculations have revealed the fact that the combined viruses of tomato and potato mosaic may be passed through tobacco and pepper, in both of which mosaic only develops, and yet produce streak on tomato. In other words, tobacco and pepper can act as intermediate hosts. Similar experiments with other Solanaceous plants are now in progress.

DISCUSSION

The results of the experiments embodied in this paper lead to the conclusion that streak disease of tomato at Macdonald College belongs to the "filterable virus" group of plant diseases.

The results show plainly that the disease is of a systemic nature, since leaves and fruit, themselves not visibly affected but coming from otherwise diseased plants, can be carriers of the inoculum. The presence of bacteria in the lesions could never be demonstrated definitely in stained sections. That bacteria are frequently found associated with mosaic tissue has been observed by other writers (14). A yellow bacterial organism, which is apparently identical with *Bacillus lathyri,* has repeatedly been isolated from streak lesions, but no further evidence has been obtained to show that it is other than secondary.

The fact that streak juice is still infectious after being passed through a bacteria-proof Chamberland F filter; after being subjected to a temperature of 70° C. for ten minutes; and finally, after being treated with strengths of mercuric chloride injurious to bacteria, for one to two days, definitely demonstrates that streak disease of tomato in Quebec is not of bacterial origin.

That the disease is not solely a physiological or malnutritional one is shown by its infectious nature. Even if tomato plants are grown under conditions which are recommended as preventive measures, and then inoculated with streak juice, streak will develop in nearly 100 per cent of these plants, although only minor symptoms will be evident in some cases. To date, all attempts on the part of the writer to produce the disease in healthy plants by manipulating the environment have failed. In cases where others have produced the disease at will, it is possible that the infective agent was latent in those plants only to become potent when conditions were favorable for its development.

All previous experimental work indicated that tomato mosaic was one of the chief, though apparently not the only, contributing cause of streak. On this continent the two diseases were invariably mentioned as being present in the same plant. The results of the experiments with the combination viruses reported herein very strongly indicate that potato mosaic is the other contributing factor. More work is necessary to determine whether other mosaic diseases may function as potato mosaic.

SUMMARY

The experimental studies reported herein, under both field and greenhouse conditions, show that:

(1) Bacteria, which are found associated with diseased lesions, are not the cause of tomato streak.

(2) The disease is highly infectious and can be transmitted from plant to plant by rubbing the leaves of healthy plants with the macerated tissue of diseased plants.

(3) The disease is of a systemic nature, since leaves not visibly affected are infectious.

(4) Streak tomato juice is still infectious after passage through a bacteria-proof filter, can usually withstand a temperature of 70° C. for 10 minutes without losing its infectivity, and can withstand concentration of mercuric chloride which readily destroys bacteria and fungous spores.

(5) Air-dried material soon becomes non-infectious.

(6) Fertilizer treatments alone cannot prevent the disease under all greenhouse conditions.

(7) Streak in Quebec has not been transmitted through the seed.

(8) Transmission by aphids has been proved.

(9) Failure has attended all attempts to induce the disease by manipulation of environmental factors.

(10) Excessive humidity, high temperature, and succulent growth are predisposing factors. Therefore control measures should consist of guarding against all predisposing factors, keeping the greenhouse free from all volunteer Solanaceous plants, and treating regularly for insect carriers.

(11) Tobacco may serve as a carrier of streak.

(12) A combination of potato mosaic and tomato mosaic viruses, when inoculated into tomato, produces symptoms identical with those of true streak, and all evidence indicates that these combined viruses are the true cause of the trouble.

DEPARTMENT OF BOTANY,
MACDONALD COLLEGE,
MCGILL UNIVERSITY,
P. Q., CANADA.

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ANTHRACNOSE OF PEPPER (CAPSICUM ANNUUM L.)[1]

B. B. HIGGINS

WITH PLATES XXII AND XXIII

The first published report, which I have been able to find, of an anthracnose disease of pepper was made by Halsted (2) in 1890. The disease was attributed to *Gloeosporium piperatum* E. & E. In the same report another anthracnose of pepper is mentioned and attributed to *Colletotrichum nigrum* E. & H. A somewhat fuller description of this latter species is given in another article (3) published during the same year. Later, as a result of cross inoculation work with anthrocnose fungi from several host species, Halsted (5) apparently became doubtful as to the real identity of the pepper *Gloeosporium*.

In 1898 Miss Stoneman (12) obtained the perithecial stage in single spore cultures of what she supposed to be *Gloeosporium piperatum* E. & E. and named the fungus *Gnomoniopsis piperata* n. gen. and n. sp.

Later H. von Schrenk and Spaulding (10) changed the generic name to *Glomerella* because the name *Gnomoniopsis* had been used by Berlese for an entirely different group of Ascomycetes six years previous to Miss Stoneman's publication.

Shear and Wood (11), after studying the perithecial and conidial stages of anthracnose fungi from a large number of host plants, drew the conclusion that those from the majority of the hosts (including pepper) were identical and should be referred to *Glomerella cingulata* (Stoneman) von S. and S.

During recent years several severe outbreaks of pepper anthracnose have been attributed to *Colletotrichum nigrum:* but, so far as I can learn, no experimental evidence as to the relation of this organism to the decay has ever been published.

Dastur (1), in reporting a study of pepper anthracnose in India and Burma, says of *Gloeosporium piperatum* E. & E. and *Colletotrichum nigrum* E. & Hals.: "These fungi are considered to be identical and to be the conidial forms of *Glomerella cingulata* (Stoneman) Spald. and von Sch."

The present study was begun during the fall of 1919 for the purpose of determining the mode of infection and the means by which the organism or organisms live over winter. Observations indicated that, in fields where pepper had been grown for two or three years and diseased material allowed

[1] Paper number 19, Journal Series, Georgia Agr. Exp. Station.

to rot in the field, the disease was little, if any, worse than in fields where pepper had never been grown before. Preliminary inoculation tests indictated that at least some forms of *Gloeosporium* occurring on pepper fruits were not actively parasitic. They were not capable of infecting sound fruits.

Practically all inoculation tests previously reported were made on wounded fruits. Dastur (1) stated that the fungus was able to enter through the unbroken cuticle, but few details of these inoculation tests were given and one could not be certain that the inoculated fruits were free from cracks or small punctures. Following our failure to produce infection on sound fruits, these cracks came under suspicion as the points of attack by the fungus. Selection for the purpose of developing a type of Pimento less subject to cuticle cracks was actually begun in 1920, but was abandoned when later results showed these cracks to be of little or no consequence in the development of fruit rots.

ISOLATIONS AND INOCULATION EXPERIMENTS

The first isolation of *Gloeosporium* was made during the summer of 1919 from a partly decayed ripe Pimento pepper fruit. A great many inoculations were made on Pimento fruits of all ages with both spores and mycelium of the fungus, but only a few infections resulted on wounded fruits and none on those not wounded. In all cases where infection occurred, the decay did not spread to any appreciable extent until the host fruit began to ripen.

During the following spring some Chili pepper growing in the greenhouse became badly diseased. Comparatively small spots, usually one to two centimeters in diameter, appeared on the young green fruits as well as on the ripe fruits. The spots were sunken, circular to oblong in outline, only slightly changed in color, and bearing large orange-yellow acervuli usually arranged concentrically on the spot. The conidia were slightly larger and more uniform in size than those isolated the previous year. Both the spots and the associated organism seemed to correspond in every way with the description of *Gloeosporium piperatum* E. & E. as given by Halsted. Elongated, grayish spots were also produced on the branches and main stem of the plants, these in some cases girdling and killing the entire plant.

Spores were scraped from one of the fruit spots, suspended in water, and applied with a sterile camel's hair brush to 5 green pods of Chili, 5 Pimento, and 6 Bell peppers. The inoculated pods, with a like number of checks, were wrapped with moist absorbent cotton and kept moistened for 5 days. At the end of 7 days the cotton was removed. At this time spots were evident on some of the inoculated pods, and, by the tenth day,

typical anthracnose spots with orange yellow acervuli were found on four Chili, one Bell, and two Pimento pods. One of the Pimento pods had 30 spots and the other about a hundred. None of the checks were diseased.

A single spore culture was obtained from a diseased Chili fruit and was later used in making a large number of inoculations. Infections were produced readily on young fruits less than two inches long, but less frequently on older fruits after the cuticle had begun to thicken.

During the fall of 1921 a *Gloeosporium* was isolated from a pale green spot on the cotyledon of a young seedling from commercial pepper seed. The morphological and cultural characters were similar to those of the form from Chili fruits, and successful inoculation of young pepper plants by spraying with a spore suspension and of young unwounded fruits by the previously described method proved it to be actively parasitic.

During the summer of 1924 an anthracnose, produced by what appeared to be the same species of *Gloeosporium,* was very severe on a small plat of Bell pepper in the field. More than 50 per cent of the fruits were destroyed. During periods of moist weather it was difficult to find a sound ripe fruit. Single spore cultures were obtained from a diseased fruit; and conidia from these cultures were used in inoculating approximately 100 fruits of all ages. The spores were suspended in water and applied by various methods over the surface of the unwounded fruits, which were then wrapped with moist absorbent cotton and kept moist as in previous experiments. At the same time a similar number of uninoculated fruits were wrapped and kept moist as checks. About 75 per cent of the inoculations were successful. Those inoculated before the cuticle of the fruit had thickened, or after they had turned red, gave almost 100 per cent infection, but the nearly mature green fruits were resistant and rarely infected. This is doubtless due to the mechanical resistance of the thick cuticle against the entrance of the germ tube. None of the checks became infected.

About the same time, a *Glomerella* (apparently *G. piperata* (Stoneman) von Sch. & S.) was isolated (single ascospore cultures) from a pepper fruit from another field. Both conidia and ascospores from these cultures were used in inoculating 50 fruits by the same method used in the above described experiments, except that a few of the fruits were wounded. Three of the wounded ripe fruits developed spots; but none of the wounded green fruits and no unwounded fruit, either ripe or green, showed any evidence of infection.

These inoculations with *Glomerella* were made on greenhouse grown plants from the same lot, at the same time, and under identical conditions as some of the inoculations with the parasitic form from Bell pepper. The *Glomerella* is evidently only very weakly parasitic, a wound parasite of ripe fruits.

Gloeosporium was again found in a field of Paprica pepper during the summer of 1925. The fungus was isolated and proved to be actively parasitic by its ability to infect sound fruits. Twenty-one Bell pepper fruits of various ages were inoculated without wounding, by methods previously described, with conidia from a single spore culture. Eight, or 38 per cent, developed typical anthracnose spots. At the same time 26 similar fruits were inoculated with conidia from a freshly isolated culture of *Glomerella piperata*. None of these showed any evidence of infection.

Further positive evidence of the parasitism of the form from Chili pepper fruits was gained from a histological study of inoculated spots.

HISTOLOGICAL STUDY OF THE DISEASE

Conidia from a culture of the actively parasitic *Gloeosporium* from Chili pepper were suspended in sterile water, to which a little powdered chalk had been added as a marker. With a sterile pipette droplets of this spore suspension were placed on the surface of young pods of Chili pepper. The pods were then wrapped with moist absorbent cotton. At the end of two, three, five, and seven days some of the inoculated spots were cut out and killed in Gilson's mercuric chloride solution. The material thus obtained was embedded in paraffin, sectioned, and stained with various stains.

At the end of 48 hours the spores had germinated and had formed appresoria which clung to the host cuticle, but no cases of actual infection were found. In material killed the third day many infection tubes from the appresoria had passed through the cuticle and had begun to kill the surrounding cells (Pl. XXIII, Figs. 13–15). By the fifth day the fungous mycelium had spread extensively through the host tissue, large areas of the host tissue were killed, and in one case an acervulus was beginning to form (Pl. XXIII, Fig. 16). The material killed the seventh day was not well fixed and could not be used.

For a more general study of the acervuli and of the more advanced stages of decay of the host tissue, naturally infected spots in various stages of development were killed, sectioned, and stained by similar methods.

In many cases the host cuticle directly beneath the appresorium was changed in some way so that it was stained an intense black by Haidenhain's haematoxylin when other portions of the cuticle remained almost colorless. Apparently this occurred only when the appresorium was beginning to germinate. Usually, in such cases, one or more of the epidermal cells directly beneath the appresorium were dead, as indicated by the coagulation of the protoplasm and disintegration of the cell nucleus (Pl. XXIII, Fig. 13). Appearances indicate that the germinating appresoria secrete an enzyme, or other substance, which dissolves, or at least softens, the cuticle, and also some toxic substance which penetrates and kills the neighboring host cells.

As the appresorium germinates, a slender infection tube from the under surface passes directly through the cuticle, passing sometimes directly into an epidermal cell and sometimes between cells. As infection proceeds, the host cells are killed a distance of two to three, and sometimes more, cells ahead of the advancing hyphae. The middle lamella very quickly disappears, and the cell wall of the host is split into very thin lamellae (see cells in Pl. XXIII, Figs. 16 and 19), the number depending upon the thickness of the cell wall. In the thick-walled cells near the epidermis, as many as 8 lamellae could be counted in the partly decayed cell wall, while in the large thin-walled cells of the interior there were usually only two.

Penetration of the cell walls by the fungous hyphae appears to be effected partly by the dissolving action of an enzyme and partly by mechanical force. In early stages of decay no indications of mechanical force are found; but in later stages the elements of the cell wall are usually pushed out of place (Pl. XXIII, Fig. 17), indicating that considerable force is exerted by the advancing hyphae. Cutinized and lignified membranes are attacked very slowly. Hyphae enter the lignified cell walls of the vascular bundles through very small openings (Pl. XXIII, Fig. 18), apparently always through pits. The middle lamella soon disappears, but the lignified portions remain apparently intact when the surrounding tissues have entirely distintegrated. Corrosion of the cutin is quite evident where hyphae are in direct contact with it, but this action is only local and very slow. In mature pepper fruits the cell walls are cutinized to a depth of 3 to 8 cell layers, the cutin being deposited between the cells. In old anthracnose spots on such mature fruits, the cellulose walls are disintegrated and split into their elemental lamellae while held together by the apparently unchanged cutin deposit (Pl. XXIII, Fig. 19).

The greatest vegetative growth of the fungus is within the inner soft tissue with thin cell walls; but, when acervuli begin to develop, the epidermal cells become packed with mycelium. As the conidiophores develop, the outer wall of the epidermal cell is pushed up with the cuticle. Sometimes conidiophore layers are again formed in the underlying cells, and the first layer is pushed up to become a part of the loose covering of the new acervulus.

The mycelium also pushes into the internal cavity of the fruit and attacks the placentae and seed, often killing the embryo. Whether or not the mycelium remains dormant in the coat of viable seed has not been determined; but it has been isolated from cotyledons of seedlings from seed of a diseased Chili pod that had been soaked two minutes in a 1–1000 solution of mercuric chloride.

OTHER ANTHRACNOSE ORGANISMS ISOLATED FROM PEPPER

In testing various lots of commercial seed, a large percentage of the seedlings from some lots developed anthracnose spots on the cotyledons or hypocotyls, when grown under moist conditions. On some the acervuli had abundant setae, while others showed no setae whatever. Isolations were made from a number of these that appeared to be different.

Our experience with the first organism isolated, indicating that it was not actively parasitic, together with observation in the field, led to the suggestion that perhaps we were dealing with several closely related organisms. During the summer and fall of 1921 a systematic search was made in the pepper fields for pepper pods bearing anthracnose spots of peculiar appearance. Notes were made as to the general appearance of the spots and as to the microscopic characters of the causal organism. Single spore cultures of the organism present were isolated from each spot thus noted. The cultures were numbered and labeled *Colletotrichum* or *Gloeosporium* in accordance with the presence or absence of setae in the acervuli of the original spot.

Including those obtained from seedlings, 19 cultures were isolated and kept growing on culture media until intensive study and comparisons could be made. This was not possible until the summer of 1923, when the cultures had been on artificial media one and a half to three years and two had been lost.

The 17 remaining cultures were grown on cornmeal agar, cornmeal mush, oatmeal agar, glucose agar, steamed bean pods, steamed pepper stems, and steamed tomato stems. Further isolations were made during the course of this study in 1923 and these were used also in some of the comparisons.[2]

The glucose agar had an initial reaction of pH 7.2. An indicator, brom thymol blue, was added before sterilization. Glucose broth was also prepared in the same manner. On both media the H-ion concentration at first increased to the yellow color of this indicator and then decreased to the full blue color of the indicator. There was considerable variation in the time required for these changes in reaction; but the general course of the change was the same in all cultures, indicating that the reaction would probably have little value in separating species in this group of fungi.

On the other media the color, luxuriance, and general appearance of the mycelial growth, the presence or absence of acervuli, their color when present, the size and shape of conidia, the presence or absence of setae, the presence of sclerotia, and the presence or absence of perithecia were noted and tabulated for easy comparison.

[2] Most of the comparisons were made by Dr. Adeline Ames while working as a special assistant during the summers of 1923 and 1924. Thanks are due to her for careful notes on the cultures and also for some of the drawings in Plate XXII.

In general the cultural characters were not sufficiently distinct and constant to be of much descriptive or diagnostic value. Some of the more distinct forms did have distinctive characteristics in culture, but these were less striking than the morphological and other characters on the host plant. For instance *Colletotrichum* No. 3 and No. 5 produced in young cultures on cornmeal a burgundy red color in the substratum, but this color gradually disappeared and was not distinctive in older cultures.

The presence or absence of setae was not constant in a few cultures. In three originally labeled *Colletotrichum,* no setae could be found in some cultures and two labeled *Gloeosporium* occasionally produced what appeared to be setae. It seems doubtful that they should be classed as true setae in either case. The spots from which some of the *Coletotrichum* cultures were obtained showed dark seta-like structures in older parts of the spots. These were generally rather elongate, much septate, thin-walled, and with a general appearance of being abortive conidiophores. Cultures from forms of a distinctly *Colletotrichum* type such as Nos. 3 and 5 (described under *Colletotrichum* sp.) always produce abundant setae. Even when conidia were found on scattered conidiophores, they were usually accompanied by one or more setae (Pl. XXII, Figs. 8 and 9).

Another, which appears to be identical with *Colletotrichum nigrum,* was isolated three different times; but in all cultures few or no conidia were produced and usually no setae. On all media tried, the cultures turned black after a few days, with innumerable small black sclerotia. Young cultures from spores usually produced conidia; but sub-cultures made by transfer of mycelium usually produced only sclerotia.

In all the cultures, the conidia produced on artificial media were slightly more variable in size than those produced on pepper fruits, but always of the same shape and general type. For instance, the conidia of *Colletotrichum* Nos. 3 and 5 were curved and had pointed ends on all media.

Two of the original cultures (one of *Colletotrichum* and one *Glomerella* from ascospore) and two of *Gloeosporium* isolated during the summer of 1923 produced perithecia in culture. Perithecia were also found quite commonly on diseased pods in the field during 1923. Upon the characters of perithecia and ascospores, however, these could be separated into two distinct groups, apparently distinct species.

IDENTITY OF ORGANISMS STUDIED

The characters of five of the forms studied were so distinct as to leave little doubt of their being separate species. The actively parasitic *Gloeosporium* isolated from spots on Chili pepper fruits in 1920, from cotyledon of seedling in 1921, from Bell pepper fruit in 1924, and from Paprica in 1925, are all apparently identical with *Gloeosporium piperatum* E. & E.

The description of this species is emended as follows:

Gloeosporium piperatum E. & E. Spots circular to elliptical in outline, 1–2 centimeters in diameter, depressed, dirty green to yellowish, turning brown in age. Acervuli formed within the epidermal or subepidermal cells, erumpent, circular to oblong, yellowish orange to salmon color; Conidia one celled, short rod-shaped, 5.4 – 6.2 μ by 15.5 – 18.64 μ, ends rounded, hyaline, with prominent nucleus showing as refractive spot in center.

Parasitic on fruits, stems, and leaves of *Capsicum annuum.*

Colletotrichum nigrum E. & H. Spots irregular in outline, indefinite, depressed, blackish; acervuli numerous, scattered over the spot, sub-cuticular, sub-epidermal or superficial, up to 100 μ in diameter, black with numerous setae; setae dark brown, septate, stout, tapering upward, 2 – 6.2 μ by 75 – 100 μ; conidia, hyaline, cylindrical, 3.5 – 5.3 μ by 18.6 – 25 μ, ends blunt. Small black sclerotia found within the host tisue and in culture media.

Wound parasite on fruits of *Capsicum annuum.*

Colletotrichum nigrum shows evident close relationship with *C. tabafica* (Hallier pro-parte) Pethybridge (9) and other sclerotium-producing species.

Colletotrichum sp. Spots irregular in outline, indefinite, depressed, dark brown to blackish; acervuli numerous, black; setae numerous, septate, stout, straight or slightly bent, tapering upward, dark brown to black; conidia one celled, hyaline, sickle-shaped, ends pointed, 4 – 5.4 μ by 18.6 – 25 μ. Small black sclerotia formed in cultures and occasionally within the host tissue.

Wound parasite on fruits of *Capsicum annuum.*

This species is possibly identical with *Colletotrichum omnivorum* Halst. (4) but apparently differs from it in size of spores and greater abundance of setae.

Glomerella piperata (Stoneman) von Schrenk & Spaulding. Perithecia scattered, partly embedded in the host tissue or in a more or less stromatic mass of hyphae, globose to oval or pear-shaped, smooth or strigose with dark green hyphae about base and occasionally about ostiole, ostiolate, sometimes slightly beaked; asci linear-club shaped; ascospores hyaline with a prominent central nucleus, linear, curved, 4 – 6 μ by 18 – 24 μ, germination usually direct without formation of cross wall.

Conidia. Spots pale straw color with dark acervuli scattered over older parts; acervuli erumpent, small, setose and dark in age; setae black, septate, tapering, few to numerous; conidia continuous, hyaline, cylindrical, 3.5 – 6 μ by 10.5 – 25 μ.

This species is probably identical with *Gnomoniopsis piperata* Stoneman, but positive identification seems well nigh impossible at the present time, since the only type material (prepared slides) of *G. piperata* now available show few of the characters of the fungus. The perithecia were described by Miss Stoneman as usually long beaked, whereas ours have rarely shown a well developed beak; but the asci and ascospores agree so closely with her description that there is little doubt in my mind as to its identity.

Glomerella sp. Perithecia scattered or in small groups, embedded in the host tissue, finally erumpent or in a more or less stromatic mass of hyphae, globose to flask shaped, usually long beaked; asci slender club-shaped, 12 by 40 – 80 μ; ascospores hyaline to smoky, straight, oblong, 6 – 9 μ by 12 – 18 μ, at first continuous but uniseptate in old perithecia and always becoming septate before germination.

Conidial stage: spot circular or often irregular in outline, indefinite, dark colored towards center; acervuli scattered, erumpent, at first pale yellow or straw colored, sometimes slightly setose in age; conidia hyaline, short cylindrical, 4 – 6.5 μ by 16 – 21 μ, continuous, becoming uniseptate before germination.

Wound parasite on fruits of *Capsicum annuum.*

The fact that several species of anthracnose-producing organisms occur on pepper pods offers a probable explanation of the very divergent opinions expressed as to the identity of the pepper anthracnose organism and its relation to anthracnose on other plants. Apparently *Gloeosporium piperatum* is not nearly so common on pepper pods as are some of the other weakly parasitic forms. The latter infect blosson-end rot spots and other wounds and are nearly always present in pepper fields. They are, therefore, the forms most frequently studied and reported as pepper anthracnose.

Often two or more anthracnose organisms and other saprophytic fungi occur on a single spot. In one case, while isolating *Colletotrichum nigrum* from an original blossom-end rot spot, one single spore culture proved to be *Glomerella piperata.* Fortunately the fruit from which the isolation had been made was saved. Upon more careful examination it was found that the two organisms could be distinguished as distinct colonies which had intermixed along the edges, and Glomerella pirithecia were present on portions of the spot.

No such mixture of organisms has been found in spots produced by the parasitic form here reported, although this also seems entirely possible.

From my own observations, I am led to doubt Miss Stoneman's having obtained the ascigerous stage of *Gloeosporium piperatum.* I have never found any indication of perithecia either in cultures of what I have here

considered *Gloeosporium piperatum* or on pepper fruits affected with this form.

Several workers have made comparisons of anthracnose organisms isolated from various hosts and upon these comparisons have based the conclusion that they all belonged to the same species. Cross inoculations have, in most cases, been made by placing the fungus in wounds on more or less fleshy fruits. Under the conditions of these experiments any such weakly parasitic, poorly specialized organism might attack almost any fleshy fruit. Such cross inoculation experiments are without serious value in determining species in such an omnivorous group of fungi.

Furthermore, any of these wound parasites may be found on wounded fruits or plant parts of other species of plants, which may account for the widespread occurrence of what Shear and Wood (11) and others have considered *Glomerella cingulata* isolated from various hosts.

The opinion expressed by Dastur that *Gloeosporium piperatum* and *Colletotrichum nigrum* E. & H. are one and the same fungus and that both are identical with *Glomerella cingulata* does not appear to be well founded. From his discussion I am led to doubt his having worked with *Gloeosporium piperatum,* and feel certain that he never worked with *Colletotrichum nigrum.* These two have so few characters in common that I do not believe that anyone, having compared them, could say that they are identical.

OVERWINTERING OF GLOEOSPORIUM PIPERATUM

With many parasitic Ascomycetes the perithecial stage matures in early spring and the ascospores serve as the principal, and often the only, source of spring infection; but, in the section of *Gloeosporium* to which *G. piperatum* is most closely related, the perithecial stage develops along with the conidia and apparently does not play any very important role in the propogation of the fungus. Possibly the ascospores are more resistant than conidia to unfavorable environmental conditions and may be able to live in the soil for a longer period. But, if *G. piperatum* has a perithecial stage, it develops so rarely as to be unimportant in disseminating the fungus.

The longevity of the conidia in soil or under field conditions has not been determined; but there is no indication that anthracnose is worse in fields where pepper has been grown for three or more consecutive years than in fields where pepper has never been grown before. Halsted (6) reported that application of decayed fruits and stems from previous crops did not increase the amount of disease. From observation I am led to believe that seed-borne spores are the most important source of primary infection.

The results of seed treatment tests and examination of seedlings previously reported (7) indicate that the spores of *Gloeosporium piperatum,* as

well as those of other fungi, become attached to the surface of the seed during the process of washing and drying and infect the young seedlings as they emerge from the seed coat. The fungus persists in rather inconspicuous spots on the leaves and stems of the plants until fruits are formed. This is probably the most important source of pod infection.

The amount of seedling infection can be greatly reduced by surface disinfection of the seed. Soaking 5 minutes in a 1–1000 mercuric chloride solution or 10 minutes in a 1–60 solution of copper sulphate followed by dusting with air slaked lime has been found effective for this purpose. Presoaking in water before either treatment increases the efficiency of the disinfectant.

SUMMARY

1. A study of pepper anthracnose has shown that several species of *Gloeosporium* and *Colletotrichum* occur on the fruits and other parts of the pepper plant. At least five distinct species have been isolated during the course of the study.

2. Of these, only one species, apparently *Gloeosporium piperatum*, is actively parasitic. This species attacks the fruits in all stages of development and occasionally produces spots and girdling of stem and branches.

3. *Gloeosporium piperatum* is comparatively rare in pepper fields.

4. The other four species are wound parasites, which enter and sometimes enlarge blossom-end rot spots and other wounds on pepper pods. These are the forms most common in the field.

5. *Colletotrichum nigrum* is a wound parasite on pepper pods. It has very distinctive characters and is not easily confused with *Gloeosporium piperatum* or any of the other anthracnose organisms found on pepper during the present study.

6. The perithecia of two species of *Glomerella* have been found on pepper pods. Both have Gloeosporium-like conidial stages.

7. The germinating spores of *Gloeosporium piperatum* produce one or more appresoria, which, after becoming firmly attached to the cuticle of the pepper pod, send infection tubes through the cuticle and into the interior of the pod.

8. The entrance through the cuticle is gained apparently by a cutin dissolving substance produced by the appresorium or by the infection tube.

9. The fungous mycelium secretes some toxic substance which kills the host cells before they are actually penetrated by the advancing hyphae.

10. The cellulose and hemi-cellulose membranes of the host tissue are readily destroyed by the fungus; but cutinized and lignified membranes are very resistant to its action, being decayed only very slowly.

11. The spores of the fungus become attached to the surface of the pepper seed during the process of cleaning and washing and attack the young seedlings as they emerge from the seed coat.

12. Surface disinfection of the seed greatly reduces the amount of seedling infection.

GEORGIA EXPERIMENT STATION,
EXPERIMENT, GA.

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DESCRIPTION OF PLATES

PLATE XXII

Drawings to illustrate morphological characters of five anthracnose fungi isolated from pepper fruits. All figures except 1 and 4 drawn to same scale with Zeiss 6 ocular and D objective, with the aid of a camera lucida.

Fig. 1. Perithecia of *Glomerella* sp. developed on steamed tomato stems.
Fig. 2. Asci of same species of *Glomerella.*
2a. Ascospores.
2b. Germinating ascospores.
Fig. 3. Conidia of same species of *Glomerella.*
3a. Germinating conidia.
Fig. 4. Perithecia of *Glomerella piperata.*
Fig. 5. Asci of *G. piperata.*
5a. Ascospores of *G. piperata.*
5b. Three germinating ascospores.
Fig. 6. Conidia of *G. piperata.*
6a. Three germinating conidia.
Fig. 7. Acervulus of *Colletotrichum* sp. developed on cornmeal agar plate.
Fig. 8. Conidia developing on scattered conidiophores on same medium.
Fig. 9. Conidia from steamed tomato stem.
Fig. 10. Conidia of *Gloesporium piperatum* from green pepper fruit.
10a. Four conidia germinating in water.
Fig. 11. Conidia and setae of *Colletotrichum nigrum* from pepper fruit.
Fig. 12. Three germinating conidia of *C. nigrum.*

PLATE XXIII

All figures drawn with aid of camera lucida, Figs. 13, 14, 15, and 18 with Zeiss No. 8 ocular and B. & L. 1.9 mm. (Fluorite) objective, Fig. 17 with No. 12 ocular and 1.9 mm. objective, and Figs. 16, 19, 20, and 21 with No. 8 ocular and B. & L. 4 mm. objective.

Figs. 13–15. Germinating conidia and appressoria of *Gleosporium piperatum* on the cuticle of a young fruit of Chili pepper 72 hours after inoculation. In 14 and 15 germ tubes have penetrated the cuticle.

Fig. 16. Section of Chili pepper fruit 5 days after inoculation, showing mycelium passing through pits from cell to cell in the cutinized area and the beginning of an acervulus within the epidermal cells. Note disintegration of host cell walls still held in place by cuticle.

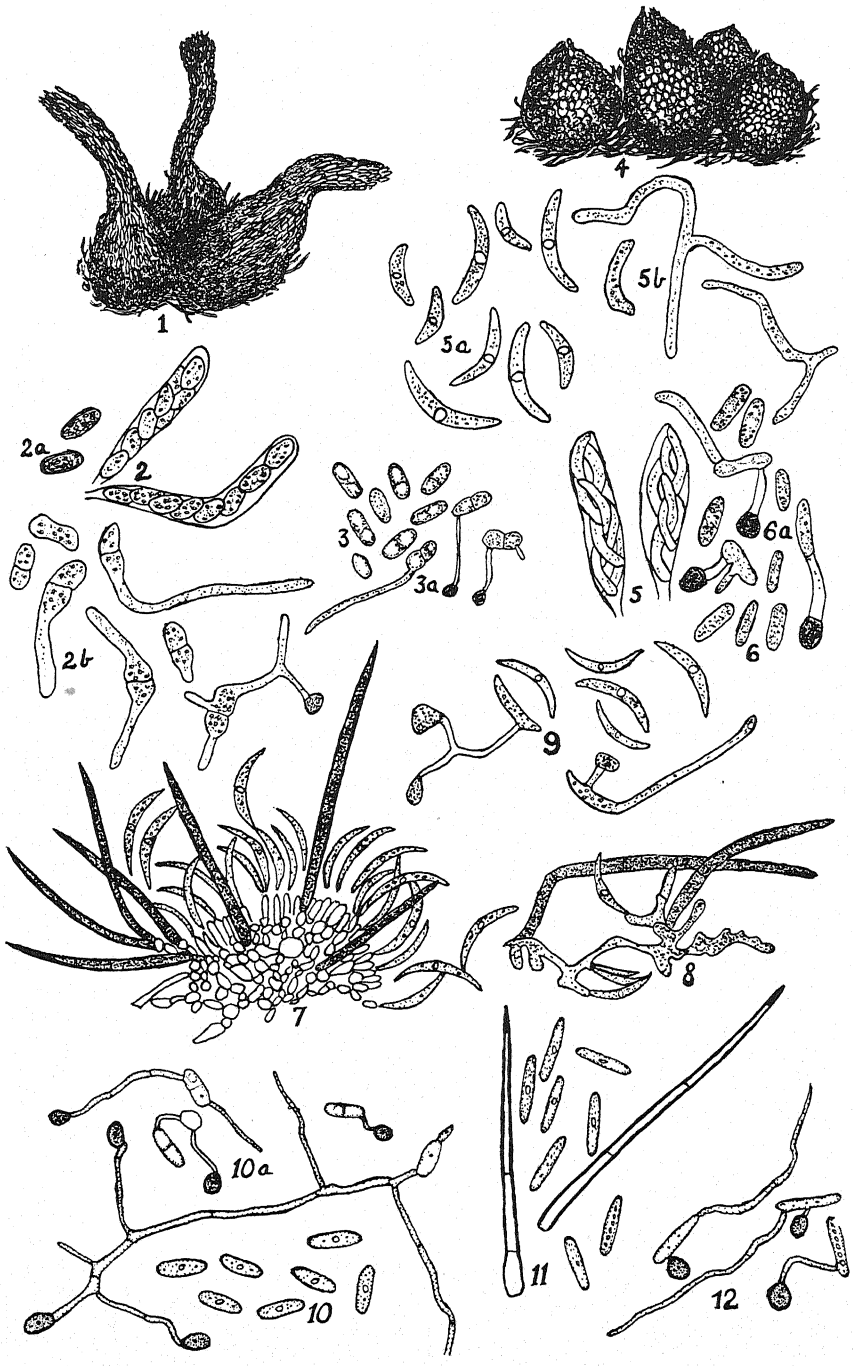
Fig. 17. Mycelium of *G. piperatum* passing through cell wall, showing dissolution of middle lamella and displacement of cellulose membrane by pressure from hypha.

Fig. 18. Mycelium in lignified ducts in a decayed area of Chili fruit.

Fig. 19. A young acervulus of *Gloeosporium piperatum* developing in the epidermal cells of a green Chili fruit, natural infection.

Fig. 20. Acervulus of *Colletotrichum nigrum* in decayed tissue of Pimento fruit.

Fig. 21. Section of sclerotium of *C. nigrum* within decayed tissue of Pimento fruit. The cells are mostly empty and dead soon after maturity.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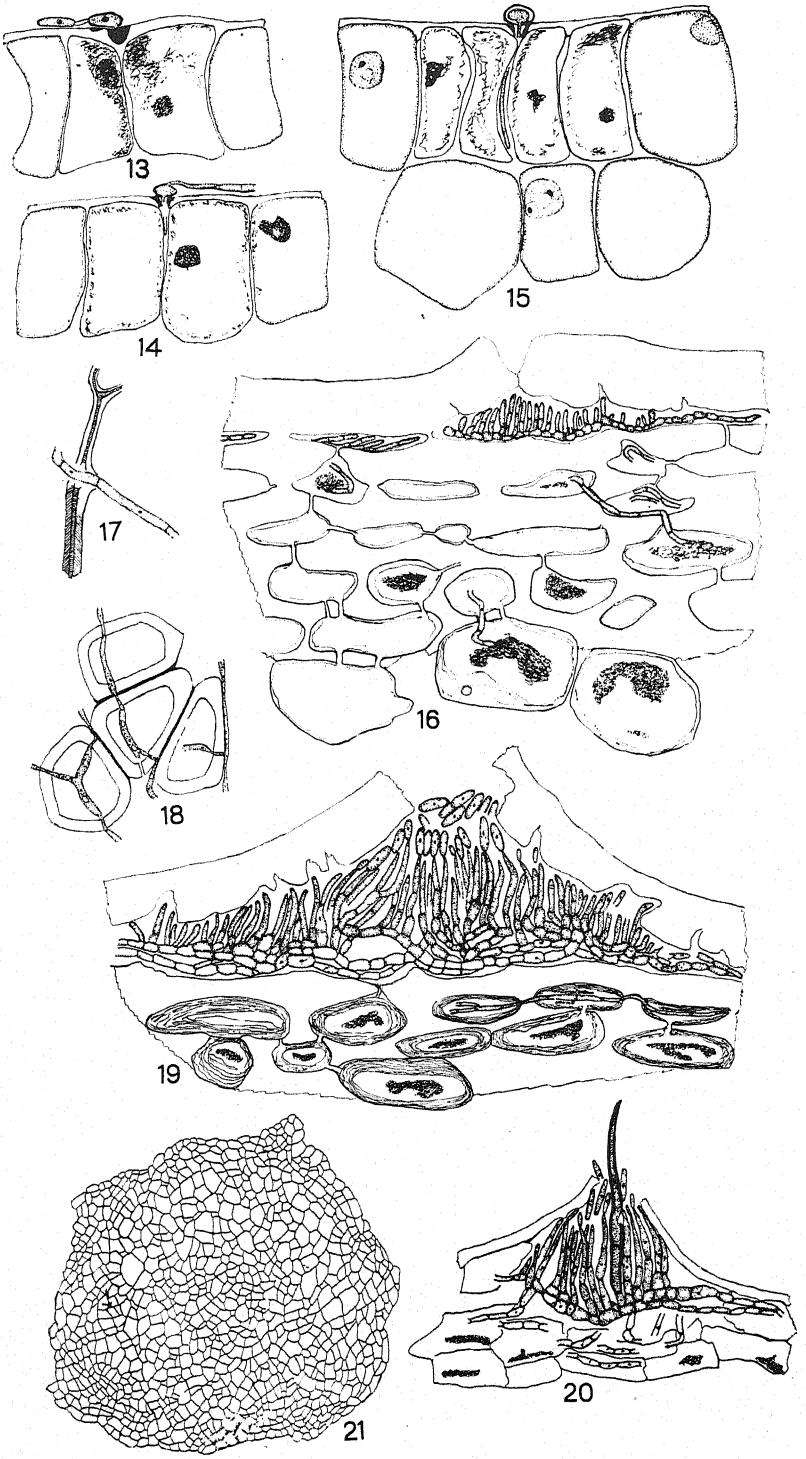
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FURTHER EXPERIMENTS ON THE CONTROL OF BUNT OF WHEAT AND THE SMUTS OF BARLEY AND OATS

R. W. LEUKEL

During the past few years much experimental work has been done on new seed treatments, both liquids and dusts, for the control of cereal smuts. Many new proprietary compounds have been developed for plant disease control, some of which have considerable merit.

From 1921 to 1924 about thirty such compounds were used in experiments at the Arlington Experiment Farm, Rosslyn, Va., in an attempt to find materials superior to bluestone and formaldehyde for the control of certain of the cereal smuts.[1] During the crop season of 1924–1925 the most promising of these compounds, together with others more recently received, were selected for further study. As the data from these studies, to a great extent, confirm those of previous investigations, it is thought desirable to publish them.

Experiments with treatments for the control of bunt were confined almost entirely to dusts, of which twenty were used. In order to test thoroughly the fungicidal properties of these dusts, Purplestraw wheat from the crop of 1924 was heavily dusted with bunt spores by mixing 1 part of bunt with 100 parts of wheat by weight. This mixture was shaken in a closed container so that the kernels were thoroughly coated with spores. The excess bunt was then removed by sifting. This procedure resulted in more heavily infested seed than probably is ever sown under ordinary field conditions. Bunt control by a compound under these circumstances, even if not perfect, should serve as an indication of its merits.

Inasmuch as the seed was very heavily bunted, the dusts also were applied quite heavily—at the rate of 8 ounces per bushel. Wheat usually will not retain more than 2 to 3 ounces per bushel, so, after the mixture had been thoroughly shaken in a closed container, the excess dust was removed by sifting. Three liquid treatments were used for purposes of comparison.

The treated seed was sown on Arlington Experiment Farm, Rosslyn, Va., October 13, 1924, at the rate of one gram per foot in 16-foot rows replicated several times. Germination tests were made by sowing 250 kernels

[1] Tisdale, W. H., Taylor, J. W., Leukel, R. W., and Griffiths, Marion A. New seed disinfectants for the control of bunt of wheat and the smuts of oats and barley. Phytopath. 15: 651–676. 1925.

from each lot of treated seed in 16-foot rows replicated four times, along with fifty similarly sown rows of untreated seed. Germination counts were made ten days after sowing. Germination tests also were made in greenhouse benches.

Infection counts were made in June, 1925. In those rows in which infection was light, all the bunted heads were counted. The average total number of heads per row was determined by counting the heads in a number of representative rows, and from this the percentages of infection were computed. In the more severely bunted rows the percentages of infection were arrived at by examining a hundred heads in each row.

The different rows were harvested and threshed separately, and the average yield in bushels per acre was computed for each treatment and for the corresponding untreated controls. The germination and infection data, together with the yields in bushels per acre, are shown in table 1.

Of the twenty dusts used, thirteen controlled bunt satisfactorily, taking the abundantly infested seed into consideration. Formaldehyde was the only treatment which completely eradicated bunt, but it reduced germination and yield considerably. Copper sulphate solution controlled bunt satisfactorily, but its effect on germination and yield also was undesirable. Five of the eight dusts furnished by the E. I. du Pont de Nemours and Company, Inc., were fairly satisfactory, but not all of these as yet are being manufactured in large quantities. Excellent results followed the use of Karasch Compounds "A" and "B," but these substances are not being made on a commercial scale. Bayer No. 3, Corona 640–S, and nickel carbonate also were fairly satisfactory. Copper carbonate, as in previous experiments, gave excellent results from the standpoint of germination, bunt control, and yield, even when it contained only 18 per cent of metallic copper. Among the commercially available dusts it seems unsurpassed for general use in bunt control.

Experiments were conducted on the control of covered smut of barley with a limited number of liquid treatments, some of which had given excellent results in previous experiments. Seed of Tennessee Winter barley, naturally infested and artificially inoculated with covered smut and contained in cheesecloth sacks, was immersed in the different solutions. After being allowed to dry, it was sown on Arlington Experiment Farm, October 10, 1924, at the rate of one gram per linear foot in 16-foot rows replicated twelve times. Germination counts were made in the field and in the greenhouse, as previously described for wheat. Infection counts were made May 14, 1925, by counting the infected heads in each row. The average number of heads per row was determined as in the case of wheat, and from this the percentage of smut was computed. These data are shown in table 2.

TABLE 1.—*Summary of data on seed germination in the field and greenhouse, bunt control, and yield in Purplestraw wheat from seed untreated or treated with different disinfectants and sown in 16-foot rows on Arlington Experiment Farm, Rosslyn, Va., October 13, 1924. Infection data taken June, 1925*

No.	Treatment	Germination		Replications	Bunt	Average yield
		Field	Greenhouse			
		(Pct.)	(Pct.)	(Number)	(Pct.)	(Bushels per A.)
1	Untreated control	62	71	13	46.5	17.4
2	Copper carbonate (C.P.)	65	78	13	0.84	27.5
3	Corona Coppercarb	71	85	13	0.65	28.5
4	Untreated control	66	70	13	54.4	17.6
5	Nichol's copper carbonate	71	84	13	0.48	28.4
6	Corona copper stearate	71	75	13	8.2	27.1
7	Untreated control	68	74	13	49.7	14.8
8	Semesan	70	84	13	0.74	25.8
9	Uspulun bolus	71	81	13	30.0	18.9
10	Untreated control	66	72	13	49.1	15.5
11	Bayer No. 3	73	88	13	1.16	26.7
12	Corona 640–S	71	77	13	1.2	26.9
13	Untreated control	67	73	13	51.5	16.8
14	Dupont No. 12	73	86	13	0.5	26.0
15	Dupont No. 13	73	83	13	26.3	25.0
16	Untreated control	68	68	13	49.5	17.7
17	Copper sulphate (dust)	63	69	13	3.0	23.8
18[a]	Copper sulphate (solution)	58	76	13	0.55	25.5
19	Untreated control	66	69	6	48.0	18.9
20	Flowers of sulphur	68	76	6	16.0	27.1
21[b]	Colloidal sulphur	70	77	6	32.0	23.9
22	Untreated control	65	67	6	38.8	19.6
23	Karasch Compound A	70	84	6	0.86	29.2
24	Karasch Compound B	70	81	6	0.1	30.7
25	Untreated control	68	62	6	44.6	19.9
26	Nickel carbonate	69	80	6	2.6	28.4
27	Dupont No. 16	70	75	6	2.4	27.6
28	Untreated control	66	64	6	48.3	19.6
29	Dupont No. 18	76	83	6	0.5	28.1
30	Dupont No. 19–B	78	83	6	22.5	23.3
31	Untreated control	67	61	6	45.1	18.8
32	Dupont No. 9	75	88	6	0.2	30.5
33	Dupont No. 30	74	80	6	28.8	25.4
34	Untreated control	67	65	4	46.5	16.6
35[c]	Formaldehyde	40	50	10	0	19.0
36	Untreated control	64	67	3	44.5	19.5

[a] 1–10 dip.

[b] 1 per cent solution, 1 hour.

[c] 1:320 for 10 minutes.

TABLE 2.—*Summary of data on control of covered smut in Tennessee Winter barley and of covered and loose smuts in Aurora oat from smutted seed, untreated, or treated with various disinfectants, and sown on Arlington Experiment Farm, Rosslyn, Va., October 10, 1924, in single 16-foot rows, replicated twelve times for barley and fourteen times for oats. Infection data taken in May, 1925*

No.	Treatment	Strength	Time	Germination		Smut
				Field	Greenhouse	
		(Pct.)	(Hrs.)	(Pct.)	(Pct.)	(Pct.)
		Barley				
1	Control	–	–	78.	90.	8.
2	Uspulun	.3	1	79.	96.	0.04
3	Germisan	.3	1	85.	93.	0.
4	Control	–	–	84.	95.	8.
5	Semesan	.3	1	84.	94.	0.3
6	Corona 640–S	.3	1	84.	91.	9.
7	Control	–	–	82.	96.	7.
8	Formalin	.3	1/2	64.	80.	0.06
9	$CuSO_4$	2.4	1/6	84.	92.	1.
10	Control	–	–	81.	94.	8.
11	Colloidal Sulphur	.5	1	78.	96.	7.
12	Water	–	1	84.	95.	15.
		Oats				
1	Control	–	–	85.	92.	8.
2	Uspulun	.3	1	76.	94.	0.
3	Germisan	.3	1	75.	96.	0.
4	Control	–	–	82.	96.	9.
5	Semesan	.3	1	79.	95.	0.07
6	Corona 640–S	.3	1	78.	96.	9.
7	Control	–	–	82.	94.	10.
8	Formalin	.3	1/2	68.	95.	0.
9	$CuSO_4$	2.4	1/10	79.	96.	0.3
10	Control	–	–	81.	97.	9.
11	Colloidal Sulphur	.5	1	76.	98.	0.7
12	Water	–	1	71.	98.	12.

Germisan at .3 per cent for one hour eliminated covered smut completely, while Uspulun and Semesan reduced it to a very slight trace. Formaldehyde also gave fair control but reduced the percentage of germination. Corona 640–S, copper sulphate, and colloidal sulphur were unsatisfactory. Soaking the seed in tap water for one hour seemed to increase greatly the amount of smut. The reasons for this are not yet definitely known.

The liquid treatments used for barley were applied also to Aurora oat for the control of loose and covered smuts. The same procedure was used as in the case of barley, and similar results were obtained, as shown in table 2. Both Germisan and Uspulun at .3 per cent strength for one hour controlled the oat smuts perfectly, while Semesan allowed only a slight trace to appear. Formalin at .3 per cent for thirty minutes eliminated the smut, but reduced the percentage of germination in the field. Colloidal sulphur and copper sulphate reduced the smut to an almost negligible amount, while Corona 640–S was worthless as a preventative for oat smuts. Soaking the seed in tap water for an hour, as in the case of barley, caused a slight increase in the amount of smut.

SUMMARY

1. Among twenty dusts used in experiments for the control of bunt, copper carbonate was found to be very satisfactory from the standpoint of germination, control, and yield, even when containing only 18 per cent of metallic copper. Its low cost and abundant supply make it the logical dust to recommend to the farmer. Several other dusts have been found equal or even superior to copper carbonate for bunt control, but their high cost or limited supply at present prevent their extensive use.

2. Germisan, Uspulun, and Semesan, in the order named, were most effective for combating covered smut of barley and the covered and loose smuts of oats. Formaldehyde controlled these smuts but caused some injury to seed.

3. Soaking oats and barley in water before sowing seems to increase the amount of smut.

OFFICE OF CEREAL INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENR OF AGRICULTURE.

THE RELATION OF SOIL TEMPERATURE AND SOIL MOISTURE TO THE DEVELOPMENT OF HEAD SMUT OF SORGHUM[1]

J. J. CHRISTENSEN
WITH ONE FIGURE IN THE TEXT

INTRODUCTION

From field observations and experiments covering a period of years, it appeared that soil temperature and soil moisture determine to a great extent the occurrence and severity of head smut of sorghum, caused by *Sorosporium reilianum* (Kühn.) McAlpine. For this reason the writer made experiments to determine the effect of these two factors on the development of the disease.

METHODS

Soil temperature experiments were made in constant temperature tanks. The water in these tanks is heated by electric heating elements enclosed in waterproof brass containers. These are situated a few inches above the bottoms of the tanks. The temperature is regulated by thermostats devised and manufactured by the Specialty Manufacturing Company of St. Paul, Minnesota. These have proved more satisfactory than the generally used relay thermostats. They are operated by the contraction and expansion of bulbous discs containing ether, and immersed near the surface of the water in the tanks. The electrical contact is made and broken by the tilting of a sealed glass tube, containing nitrogen, a few cubic centimeters of mercury, and the electrodes.

The soil cans used for these tests were 20 cm. in diameter and 44 cm. deep. Each can contained the same quanitity of soil, the upper level of which was 3–4 cm. below the water level in the tanks, and was covered with 2–3 cm. of granulated cork.

Two different lots of soil were used. In 1924, heavily infested soil was obtained from a field at Waconia, Minnesota. In 1925, a black loam from University Farm, St. Paul, Minnesota, was used. In both trials three parts of soil were mixed with one part of sand. Smut spores and cultures of the organism were thoroughly mixed with the soil before the cans were filled.

[1] The writer is indebted to Dr. E. C. Stakman for valuable suggestions and helpful criticisms.

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In 1924, six tanks, each with four cans, were maintained at temperatures as follows: 16°, 21°, 25°, 28°, 31°, and 34° C. In 1925, seven tanks, each with six cans, were kept at the following temperatures: 12°–16°, 16°, 20°, 24°, 28°, 32°, and 36° C. In this last test, the soil in half the number of cans in each of the temperature tanks was adjusted to low and the other half to high moisture content. On an oven dry basis the moisture contents were approximately 15 and 25 per cent respectively. The moisture content of the soil in different tanks fluctuated somewhat, but in every

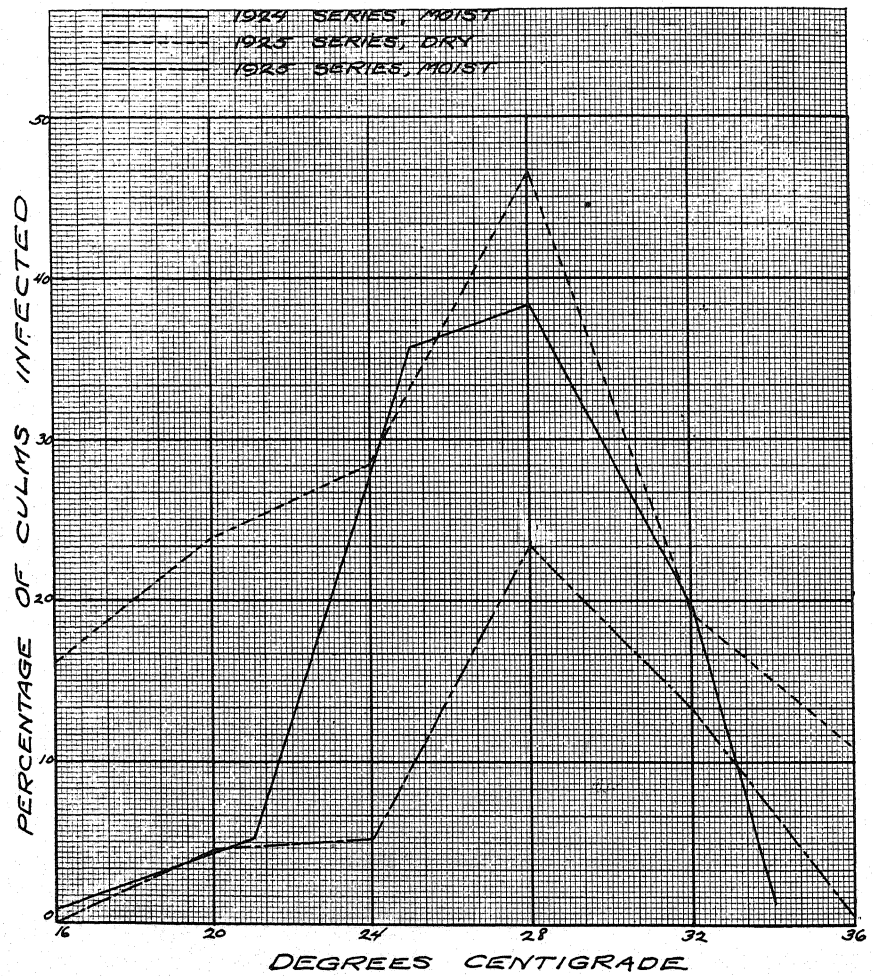

FIG. 1. The influence of soil moisture and soil temperature on infection of Minnesota Amber cane by *Sorosporium reilianum*.

case twice as much water was added to the cans in the wet series as to those in the dry series.

There was some variation at the lower temperatures: on two separate occasions the tanks kept at 16° and 20° C. varied two degrees for a few hours. The temperature of the coldest tank gradually rose from 12° to 16° C. before the completion of the three-weeks' experiment. However, the soil temperature seldom deviated more than one-half degree from those given.

The aerial parts of all the plants in a given test were exposed to similar atmospheric conditions. After the plants had developed from two to four leaves, they were transplanted to the field, where they matured. Smut counts were made in September.

Two selfed lines of Minnesota Amber sorghum, improved by Dr. J. J. Willaman of the Agricultural Biochemistry Division, University Farm, St. Paul, Minnesota, were used in all the experiments.

RESULTS

The results obtained in 1924 and in 1925 agree very closely with each other. They tend to prove that the interaction of soil moisture and soil temperature determines whether or not infection occurs, and the severity of the attack. The results are summarized in tables 1 and 2.

TABLE 1.—*The influence of soil temperature on infection of Minnesota Amber cane by Sorosporium reilianum in 1924*[a]

Temperature in degrees C.	Total number culms	Number culms infected	Per cent culms infected
16	141	1	0.71
21	57	3	5.26
25	92	33	35.87
28	73	28	38.35
31	149	29	19.46
34	107	1	0.93

[a] Moisture percentage not determined.

It is evident that *S. reilianum* can infect sorghum seedlings at temperatures ranging from 16° to 36° C. The results indicate also that the infection was not favored either by relatively high or low temparatures. From tables 1 and 2, and figure 1, it is evident that rather high soil temperature (20°–30° C.) during germination of the seed and emergence of the seedling favor infection. The highest percentage of infection was obtained at 28° C. The upper temperature limit for infection is probably near 36° C. and the lower limit apparently is not much below 16° C.

From table 2 and figure 1, it is obvious that low soil moisture (15 per cent on an oven dry basis) was much more favorable for infection than high soil moisture (25 per cent). In the moist series no smut developed at 16° C., and it was materially reduced at other temperatures. The minimum temperature for infection varies with the amount of moisture in the soil.

It is interesting to note that the thermal range for infection coincides fairly closely with that for spore germination[2] and growth of the pathogene in culture. Potter found that the optimum temperature for growth of the organism in artificial culture was between 28° and 30° C.

TABLE 2.—*The influence of soil moisture and soil temperature on infection of Minnesota Amber cane by Sorosporium reilianum in 1925*

Temperature in degrees C.	Moist series[a]			Dry series[b]		
	Total no. culms	No. culms infected	Per cent culms infected	Number plants	Total no. culms	Per cent culms infected
12–16	48	0	0	35	1	2.82
16	35	0	0	111	19	16.21
20	288	13	4.51	247	59	23.89
24	272	14	5.15	276	73	26.45
28	171	40	23.39	234	109	46.50
32	188	25	13.29	235	45	19.15
36	217	1	0.46	202	22	10.89

[a] 25 per cent moisture on oven dry basis.
[b] 15 per cent moisture on oven dry basis.

There was no correlation between the rate of germination of the sorghum seed and the percentage of smut which developed. Seeds kept at 16° C. required from 10 to 14 days to emerge, while those kept at 28° C. appeared above the surface in 3 or 4 days. Above 30° C. the time for germination was shorter than at 28° C. Seeds germinated most rapidly at 34° and 36° C., and the plants grew best at 28° to 36° C. These results seem to indicate that soil temperature and soil moisture determine the amount of smut by affecting the pathogene directly, rather than indirectly by influencing the host plant.

A low percentage of infection appears to be correlated with low temperature and high precipitation. The variation in soil moisture and soil temperature at planting time no doubt explains the differences in the severity of the disease in the same fields in different seasons. The optimum temperature for germination of spores, production of sporidia, and for infection of sorghum seedlings seldom occurs in Minnesota at planting time.

[2] Potter, Alden A. Head smut of sorghum and maize. Jour. Agr. Res. 2: 339–371. 1914.

Undoubtedly this is one of the most important factors in reducing the severity of the disease in those localities of the state where sorghum is grown intensively.

SUMMARY

1. A preliminary study was made, under controlled conditions, of the effect of soil moisture and temperature on the development of head smut of sorghum.

2. Seedlings of sorghum became infected in dry soil at temperatures ranging from 16° to 36° C. In moist soil no infection occurred at 16° C. The amount of infection was greatly reduced toward the two extremes of soil temperature.

3. The optimum soil temperature for infection was 28° C. The minimum, and probably the maximum, fluctuates with the percentage of moisture in the soil.

4. High soil moisture materially reduced the percentage of smutted plants at all temperatures and also narrowed the thermal range for infection.

5. The results indicate that soil temperature and moisture during the period of germination and emergence of sorghum plants determine to a large extent the severity of the disease under field conditions.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY FARM, ST. PAUL, MINNESOTA.

FRUITLET BLACK ROT DISEASE OF PINEAPPLE

HENRY D. BARKER

WITH TWO FIGURES IN THE TEXT

During the summer of 1924 the writer's attention was called to a disease occurring in pineapples at Cap Haitien, Haiti.

Externally the diseased fruit appears to be quite normal. During the past year several thousand fruits have been cut in an intensive study of this condition. The results indicate that in certain desirable commercial varieties, such as the Smooth Cayenne, the proportion of diseased fruits may run as high as 50 to 75 per cent during certain seasons of the year. Unfortunately the disease is apparently more severe during the summer season, which is also the principal harvest season of the fruit. A second, and generally much smaller, crop of fruit is produced during the winter months, when the disease may be less severe. Available literature indicates that the disease is unknown in the pineapple region of Hawaii. Nowell[1] describes what is apparently the same disease as occuring in various parts of the West Indies, and says that the existence of this trouble has a serious reaction on attempts to establish the pineapple industry.

The most conspicuous symptoms that we have observed are briefly as follows:

A cut fruit may reveal one or many affected fruitlets in which there are dark brown or blackish spots (Fig. 1). The diseased tissue is typically dry and more or less hard. This is so marked in severe cases that diseased fruits can be distinguished as they are being cut in two. In many cases the disease appears to commence in the placentae and extend in dark, somewhat granular radiations. A portion, or all, of the inner surfaces of the individual fruitlet may be affected, or the entire fruitlet may be blackened throughout. Even in the more severe cases, it appears to be a fruitlet rot rather than a general rot of the entire fruit.

In general, the disease does not appear to affect the connective tissue. In certain cases, however, the fibrovascular bundles in the core of the fruit are distinctly browned (Fig. 1). This peculiar rot does not manifest itself in the green or immature fruit, or even in immature fruitlets sometimes found near the top of a ripe fruit. It develops during the process of ripening, but does not spread in storage or after-ripening. A disease

[1] Nowell, W. Diseases of crop-plants in the Lesser Antilles. West India Committee, 14 Trinity Sq., London. 349–350. 1923.

fruit may be kept until yeasts and other organisms have completely fermented and decayed it, with no extension of the blackened areas. This disease must not be confused with the several somewhat similar, but less serious, fruitlet spots or rots that occasionally occur in pineapples. The

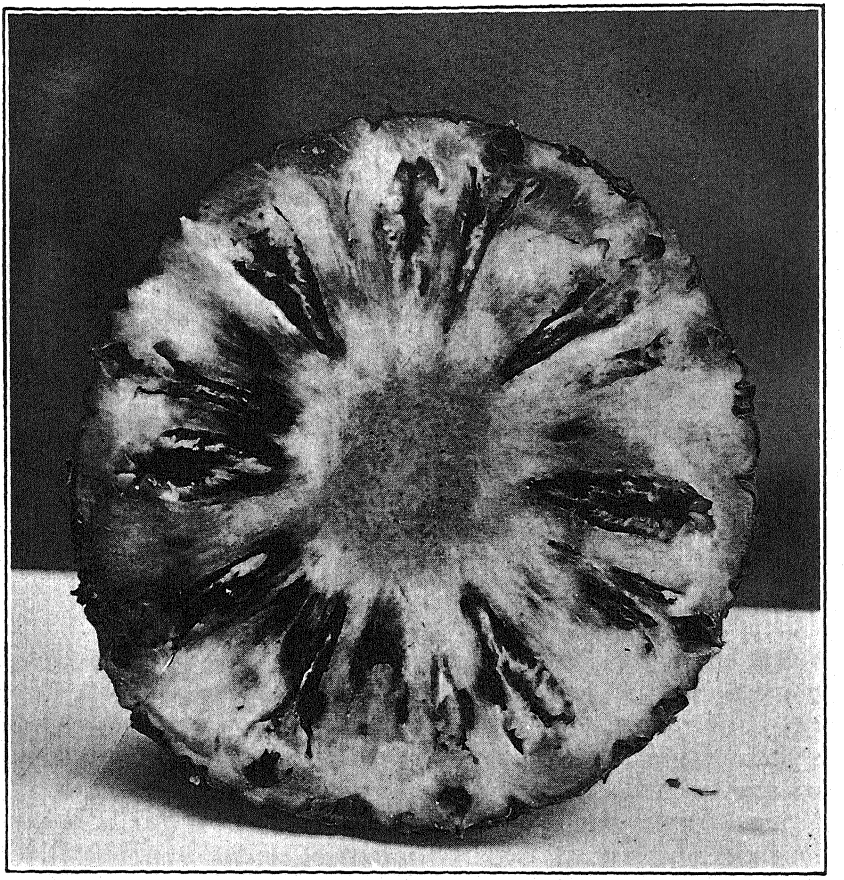

FIG. 1. Photograph of cross-section of fruit affected by fruitlet black rot. Natural infection, unusually severe. Note blackened fibrovascular bundles.

evidence indicates that unusually large fruits are more subject to attack than are the smaller ones. No external symptoms of the disease have been discovered. However, in a known, but apparently undescribed, disease, a "gummosis," in which amber-like gum or waxy secretion is exuded from the eye, seems to be a related phenomenon.

Under natural conditions in the field here, gummosis is rare, and the fruitlet underneath the gummed eye may or may not be blackened. How-

ever, when the fruitlet black rot is produced artificially by needle puncture, gummosis is a quite commonly associated symptom. This possibly may be accounted for by the fact that, under natural conditions, there is no apparent connection between the affected part of the fruitlet and the external surface of the fruit. The greater amount of inoculum and the greater injury to the tissues during artificial inoculation also may be important factors.

Although the disease has occurred in the West Indies for some 20 years, apparently very little is known concerning its actual cause and nature. From the work of Howard, in 1901, and of other mycologists of the Imperial Department of Agriculture of the West Indies, Nowell concludes: "A species of *Penicillium* is uniformly found to occupy the spots, fruiting in small cracks and in the cavity of the ovary.

"The beginning of the spots, and the entry of the fungus into the tissues is associated, commonly at least, with perforations in the tough wall of the old floral chamber (the eye) near the insertion of the style. Species of *Penicillium* are generally present on the withered floral organs, and it is regarded as probable that the fungus depends for access to the pulp, on punctures made by insects or mites inhabiting the enclosed floral chamber."

Under conditions in Haiti *Penicillium* also has been found to be almost universally present on the old withered floral organs in the "eye," in both diseased and healthy fruits. However, within the tissue of the diseased fruit *Penicillium* is rarely found, except in the more advanced stages, when it and a great variety of secondary filamentous fungi, yeasts, and bacteria become common.

In plates made from the earliest stage of the disease, the organism most commonly obtained was a pale yellowish bacterium with rugose colonies. Inoculations of this organism into the fruitlets, by needle punctures, were made in the field on fruits that were judged to be about 10 days from maturity. The first inoculations were made through the floral chamber or eye. These were then sealed with grafting wax. Practically 100 per cent infection was obtained both in the inoculated fruits and in the checks which had been punctured and sealed. In the second series of inoculations the surface of individual fruitlets was disinfected with 1–1000 bichloride of mercury, punctures were made from the outside and immediately sealed with grafting wax. Care was exercised to prevent the needle from entering or puncturing the floral cavity. This method gave uniform infection in the inoculated fruits, while the checks remained healthy, that is, the percentage of diseased fruits in the checks was no greater than the average field run at that particular time. Furthermore, the diseased fruitlets occurring in the checks were distributed throughout the fruit in such a way as to suggest no relation to punctured fruitlets. The artificially produced

black fruitlet rot was typical, and characteristic of the naturally produced disease (Fig. 2). The only divergence noted was the associated "gummosis" in the case of the artificially produced disease. In some cases the disease seemed to be strictly localized, being confined to the inoculated fruitlets or sometimes spreading to a few contiguous fruitlets; in other cases it seemed to be more or less systemic, spreading to all the ripe fruitlets and causing a browning of the vascular strands in the core of the fruit. This apparent difference in reaction has not yet been explained. Reisolations of the same yellow bacterial organism were obtained from the typically blackened tissue, from the browned bundles in the core, and from the gummy exudate.

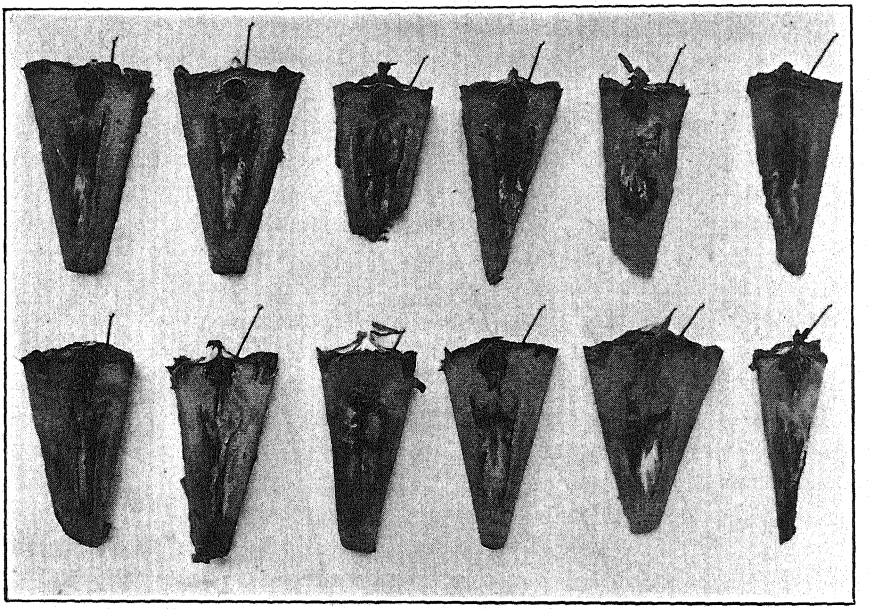

FIG. 2. Early stages in the disease. Individual fruitlets cut from cross-section of fruit in which disease was produced artificially. Note the beginning of the disease in the placentae and its development in the fruitlet.

For many reasons the problem has been, and in many features still is, a very perplexing one. Upon the completion of experiments now in progress details regarding the identity of the causal organism, attempted control measures, etc., will be published in a joint paper with Dr. G. N. Wolcott, Entomologist of the Haitian Department of Agriculture.

SUMMARY

1. A fruitlet black rot disease of pineapples occurring in Haiti is described. The disease is very severe in the improved variety Smooth Cayenne, in which 50 to 75 per cent of the fruits may be affected.

2. Cultures made from tissues in the earliest stage of the disease yielded commonly a pale yellowish bacterium with rugose colonies.

3. Inoculations with this organism produced typical symptoms of the disease, and the organism was reisolated from the inoculated tissues.

SERVICE TECHNIQUE,
DÉPARTEMENT DE L'AGRICULTURE,
PORT-AU-PRINCE, HAITI.

PERONOSPORA PARASITICA (PERS.) DE BY ATTACKING CABBAGE HEADS

T. H. THUNG

WITH ONE FIGURE IN THE TEXT

The attack of full-grown cabbage heads by *Peronospora parasitica* has not been mentioned in the literature. The general opinion is that the fungus attacks only very young plants.

The fungus not only attacks the outer leaves of the full-grown cabbage heads but also penetrates into the inner leaves, the mycelium being able to pass from one leaf to the other. In the cabbage-growing districts of Holland the fungus can easily be found throughout the year.

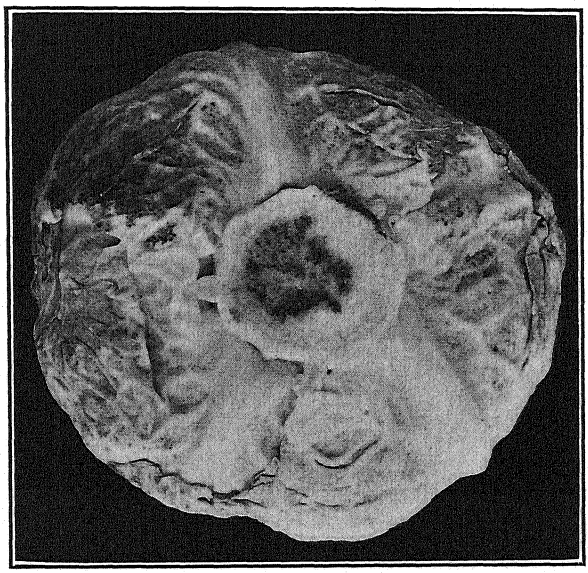

FIG. 1. Cabbage head attacked by the mycelium of *Peronospora parasitica* (Pers.) de By: the conidiophores are visible.

The old yellow leaves nearest to the ground usually are covered with irregular, circular bordered, black spots, in which the mycelium lives and on which the conidiophores are to be found.

As stated in the literature, overwintering can take place by means of oospores. Observations during the winter 1924–25, however, showed that the mycelium also can live throughout the winter in cabbage leaves, and that exposure to frost does not kill it.

Conidia may germinate and the mycelium may spread in infected cabbages stored during the winter. The longer the cabbage is stored, the more severely it is likely to be injured. The fungus even may penetrate into the parenchyma of the stem when cabbage has been stored for a long time.

The author of the present note agrees with Gäumann[1] that the *Peronospora* of *Capsella bursa pastoris* is another strain, which does not attack cabbage.

Control measures: removal of *Capsella bursa pastoris,* on which *Peronospora parasitica* also lives (mentioned as a means of control by Sorauer[2]), is not necessary. When weeding and hoeing the fields, one might at the same time gather and destroy those lower leaves of the cabbage plants which are covered with the fungus. The white cabbage varieties are more susceptible than the red ones.

Details will be published later.

INSTITUTE FOR PHYTOPATHOLOGY,
LABORATORY FOR MYCOLOGY AND POTATO RESEARCH,
WAGENINGEN, HOLLAND.

[1] Gäumann, E. Über die Formen der *Peronospora parasitica* (Pers.) Fries. Beih. Bot. Centralblatt. 35: 395–533. 1918.

[2] Sorauer, P. Handbuch der Pflanzenkrankheiten 2. Vierte Auflage, S. 213.

RHIZOCTONIA CROWN ROT OF CARROTS[1]

R. P. WHITE

WITH ONE FIGURE IN THE TEXT

Rhizoctonia crown rot of carrots was first observed in Kansas in June, 1924, in the Kaw River Bottoms immediately west of Kansas City, Kansas. Excessive rain had fallen for two weeks preceding the discovery of the trouble, bringing about saturated soil conditions and high humidity around the bases of the plants.

Dr. Charles Chupp states in correspondence[2] that this disease is quite prevalent in New York State and occasionally does considerable damage, being most injurious in localities where young carrots are sold with the tops still attached. The *Corticium* stage, he states, appears as a white weft about the bases of the leaves, almost wherever carrots are grown. This condition never had been observed in Kansas, however, until June, 1924.

The carrots affected were apparently stunted, but upon close observation this apparent stunting was found to be due to the decay of the bases of the central leaves, which either failed to develop normally or were rotted off completely. Older leaves, that were still attached to the root but were partially decayed or only slightly infected, had red leaf margins. It is questionable whether this red and bronze discoloration of the leaves is specifically associated with the infection of *Rhizoctonia* on carrots. This symptom did not appear on artificially inoculated plants in the greenhouse.

In badly diseased specimens a crown rot of the fleshy root was evident as a soft brown decay entering the crown and forming an inverted cone-shaped mass of decayed tissue. In such plants all of the central leaves had been rotted off, leaving only a few of the older leaves around the edge of the decayed zone.

Isolations were made from leaf bases and decayed crowns. The only organism constantly associated with the lesions was a species of *Rhizoctonia*, similar in gross and microscopic characters to *Rhizoctonia solani*. No cross inoculations were made to potato or other hosts. Secondary organisms were abundant, especially in the diseased roots, but none was proved pathogenic.

Both young and nearly mature carrot plants were inoculated in the crown with a small block of agar containing vigorously growing hyphal tips

[1] Paper No. 248 of the Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station.

[2] Correspondence dated October 12 and October 26, 1925.

of *Rhizoctonia*. Inoculations at first were not very successful owing to the fact, as it was found later, that relatively high temperatures and high humidity are required for infection. Infection results with plants inoculated and kept in a damp chamber for two days were inconsistent, while similarly inoculated plants never failed to develop the typical brown decay of the leaf bases and rot of the crown if left in the damp chamber for seven days (fig. 1). The typical weft of white mycelium developed around the bases of the leaves.

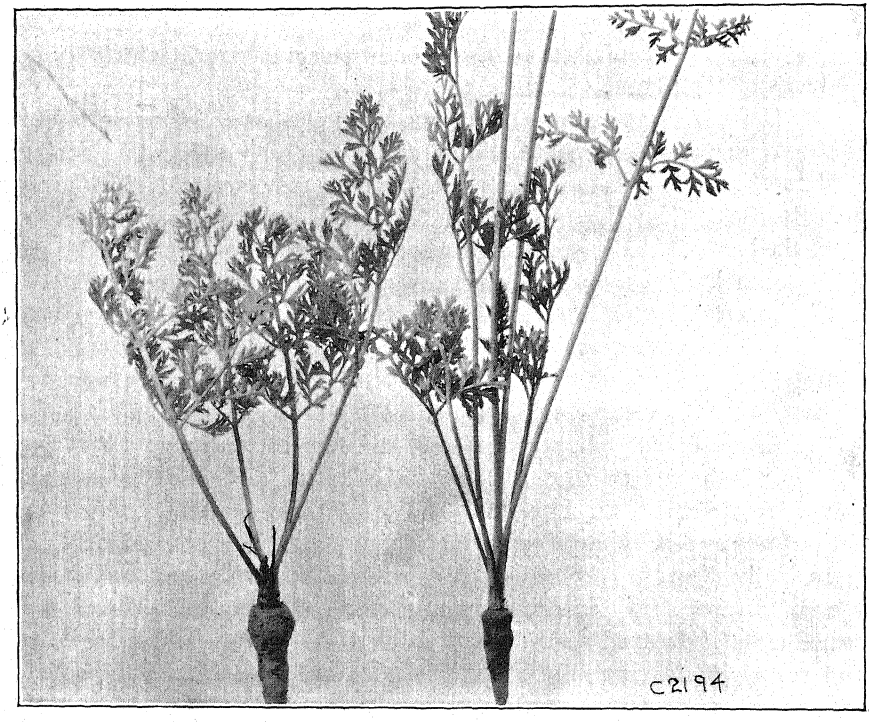

FIG. 1. Crown rot of carrots. Healthy plant on right: plant on left artificially inoculated with *Rhizoctonia sp.*

Rhizoctonia sp. is evidently a weak parasite on carrots, infecting at the crown only under conditions of high humidity. It seems as if this trouble could be avoided in wet seasons, by wider spacing of the rows to allow better circulation of the air between the plants, although no experiments upon control measures were attempted. No loss should be expected in Kansas in dry seasons or in seasons of normal precipitation.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
KANSAS AGRICULTURAL EXPERIMENT STATION,
MANHATTAN, KANSAS.

PHYTOPATHOLOGICAL NOTES

Physiologic forms of Colletotrichum lindemuthianum (Sac. et Mag.) Bri. et Cav. in the Netherlands.[1]

In the European literature on the bean anthracnose, statements can be found which indicate that certain bean varieties are immune from the disease in one place and not in another.

This fact led to the conjecture that in Europe there might be physiologic forms of the pathogene similar to those which have been found in America.

Because it was desired to compare the physiologic forms eventually to be found with those found by American investigators, the infection experiments were made with the same bean varieties used by Barrus and Burkholder of Cornell University and by Leach of the University of Minnesota, who kindly forwarded, at our request, the seed for the experiments.

Five physiologic forms have been found to date which are different from those of Barrus, Burkholder, and Leach. Two forms, which gave the same reaction on the seven bean varieties of Leach's key, and which therefore were indistinguishable by it, differed in pathogenicity for other bean varieties.

Four forms were isolated from diseased pods of *Phaseolus vulgaris* L. from different parts of the Netherlands, and one form from a pod of *Phaseolus multiflorus* Wild. The last form is characterized by its extremely rapid growth on nutrient agars.

The vulgaris forms differed in pathogenicity and in growth and appearance of the colonies on nutrient agars. All produced the ascogenous stage in the laboratory; the multiflorus form has not yet been investigated in this respect.

The experiments will be continued, and will be described *in extenso* in a later publication.—H. R. A. MULLER, Institute for Phytopathology, Laboratory for Mycology and Potato Research, Wageningen, Holland.

Banana Wilt Disease on the Island of Singapore.—Dr. Otto A. Reinking, of the United Fruit Company, writes from Singapore as follows:

"The wilt of bananas has been found on the island of Singapore in the outskirts of the city of Singapore. How widespread the disease is has not been determined. External and internal symptoms show the disease to be typical of the Caribbean banana wilt due to *Fusarium cubense*. Microsco-

[1] Received for publication September 28, 1925.

pic examination shows the presence of typical mycelial strands in the xylem tissue. The disease in all probability is due to the same *Fusarium* as is present in Central America, but this can not be stated definitely until cultures are obtained. Cultures of the organism will be made at the earliest opportunity. The disease so far has been definitely observed in the Philippines and Singapore. The disease was observed as such for the first time in Singapore on October 13, 1925.''—JOHN R. JOHNSTON, United Fruit Co., Boston, Mass.

PHYTOPATHOLOGY

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FUSARIA INOCULATION EXPERIMENTS. RELATIONSHIP OF VARIOUS SPECIES OF FUSARIA TO WILT AND COLORADO DISEASE OF BANANA

O. A. REINKING[1]

INTRODUCTION

During an investigation conducted on Fusaria in relation to banana plantations in Honduras, isolations were made from living and dead parts of diseased and non-diseased bananas; from living and dead parts of other plants growing in banana plantations and in the virgin forest to be planted to bananas; and from the virgin soil and soil in diseased and non-diseased banana plantations. Special isolations were made from banana plants affected with wilt and the Colorado disease to determine whether more than one type of *Fusarium* or any other organism was associated with these diseases. Other studies on banana wilt have shown that *Fusarium cubense* is definitely associated with the banana wilt plants and that generally no other *Fusarium* is found in typical cases of the disease.[2]

Only in two cases were other Fusaria found. One of these, an old infection, appeared to be a combination of banana wilt and Colorado disease. It was not a typical case of wilt. *Fusarium moniliforme* var. *subglutinans* Wr. and Rkg, was found in the vascular bundles of this plant. Another plant affected with banana wilt yielded *Fusarium lutulatum* Sherb. from its vascular bundles. In both of these cases the isolations were made from material that had been collected one and three days respectively before the isolations were made. This interval might well have given these doubtful fungi a chance to enter the diseased tissue from the cut surfaces. In any event, because of the extensive number of isolations made under controlled conditions from wilt diseased plants, it might well be stated from this standpoint that *Fusarium cubense* only is associated with typical cases of banana wilt. Examinations made of plants infected with the Colorado disease

[1] Pathologist, United Fruit Co.

[2] Brandes, E. W. Banana wilt. Phytopath. **9**: 339–389. 1919.

indicated that this trouble was due primarily to an abnormal cultural condition and not to fungi. Because, however, one fungus *Fusarium moniliforme* var. *subglutinans,* was commonly associated with the diseased plants and their environs, it was deemed advisable to use this organism for inoculation purposes to determine whether it was pathogenic and could produce the symptoms of Colorado disease.

FIG. 1. Inoculation experimental plot I. Concrete tanks built in the ground. Four and one-half feet square.

Soil isolations and those from banana, and other than banana plants, were made primarily to determine the distribution of *F. cubense* in diseased plantations and in virgin forests and soil. Isolations made from the soil in diseased and non-diseased plantations and virgin soil showed that there are a few closely related organisms that could be confused with *F. cubense.* These are *Fusarium oxysporum, F. oxysporum* var. *nicotianae, F. orthoceras,* and *F. orthoceras* var. *triseptatum.* Because of these closely

related forms, all of which have been found to be parasitic on plants in various parts of the world, it was found necessary to make inoculations to determine their pathogenicity and their relationship with *F. cubense* and with banana wilt.

A very careful morphological and physiological study of these and all other species collected was made in order to establish definitely their relationship. These studies culminated in the Fusarium conference held at Madison, Wisconsin.[3] Here a special study was conducted with Dr. H. W. Wollenweber on the entire collection of tropical Fusaria. Out of the collection of some 250 strains, 48 different species and varieties of Fusaria were described. These included species in practically all of the sections of the genus. Fourteen forms were described as new.[4] It might be stated here that these morphological studies of the organisms concerned corroborated very closely the results of inoculations made in the tropics. The morphological studies showed that a close relationship exists between *F. cubense* and species of the Oxysporum group. *F. oxysporum* var. *nicotianae* was found to be very closely related, although a slight difference was always present. These findings then leave one question open, and that is, whether a saprophytic form of *Fusarium* closely related to *F. cubense* can eventually, by a gradual process, develop the parasitic qualities necessary to produce disease in bananas. It is believed, however, that the following inoculation experiments, especially when correlated with isolation and morphological studies, show that there is a definite parasitic strain, *F. cubense,* that only can produce the typical banana wilt disease.

METHODS

The inoculation experiments, as planned, might well be divided into two parts: one, the preliminary experiments, carried out to determine whether the proposed method of procedure was correct; two, the main experiment.

PRELIMINARY EXPERIMENT

In the preliminary experiment, two wooden boxes and one concrete tank, all placed above the ground (Figs. 2 and 3), were used for the trials. Since the procedure was the same as that carried out in the main experiment, no detailed explanation will be given. It will suffice to say that cultures of *F. cubense* isolated from diseased plants (table 2) were used for inoculation. The results of this experiment are given in table 1.

The results shown in table 1 proved that the banana wilt disease could be produced in plants growing under the particular conditions devised for

[3] June, July, and part of August, 1924.

[4] Wollenweber, H. W., and O. A. Reinking. Aliquot Fusaria tropicalia nova vel revisa. Phytopath. 15: 155–169. 1925.

TABLE 1.—*Preliminary inoculation experiments with F. cubense*

No.	Culture used	Planted and inoculated 1922	Type of soil	Cut down 1923	Wilt disease
Box 1	R 11. *F. cubense*	Dec. 20	Fine silt	July 13	X[a]
Box 2	R 3. *F. cubense*	do	do	do	—
Tank 1	R 2. *F. cubense*	Dec. 30	do	do	X

[a] X disease present; — disease absent.

the experiment (Fig. 6). The methods of soil sterilization, planting, and inoculation were found to be successful. These are discussed under the main experiment.

MAIN EXPERIMENT

Plan of experiment. The main experiment was divided into three parts, with the idea of providing a careful check upon the different methods used, and to produce conditions that were favorable to infection. The same organisms, therefore, were tested under two, and, in four cases, under three different conditions. The first experimental plot (Fig. 1) consisted of 24 concrete tanks built into the ground. Four of these tanks were 4½ feet wide, 9 feet long, and 4 feet deep. The other tanks were 4½ feet square and 4 feet deep. A fine wire mesh fence encircled the entire plot so as to exclude rodents and other animals.

The second experimental plot (Fig. 2) consisted of 16 wooden boxes, 2½ feet square and deep, with wooden bottoms. These boxes were raised above the ground by means of pieces of timber.

The third experimental plot (Fig. 3) consisted of four concrete tanks, 4 feet square and deep, inside measurement, placed on the ground. Cement bases were provided for these boxes.

These three experimental plots gave a variety of methods, with different degrees of control from outside sources of infection, and also different growth conditions, varying from a natural condition to one not so well adapted to the growth of bananas. The boxes and concrete tanks placed on the ground gave a condition that was less apt to be influenced by ouside infection, but at the same time did not present a natural condition for growth. The concrete tanks placed above the ground, however, were large enough to grow perfectly healthy, normal plants.

Soil. Because it was known that the soil has a relation to disease production, two types of soil were used for the experiments: one a fine silt, approximately neutral soil, and the other a light, sandy, acid soil. The disease apparently does not spread so rapidly in the former soil as in the latter. The latter soil gave a condition under which the organisms used

for inoculation would have the best possible opportunity to prove their pathogenicity. The disposal of these soils in the various experiments is given in tables 2, 3, and 4.

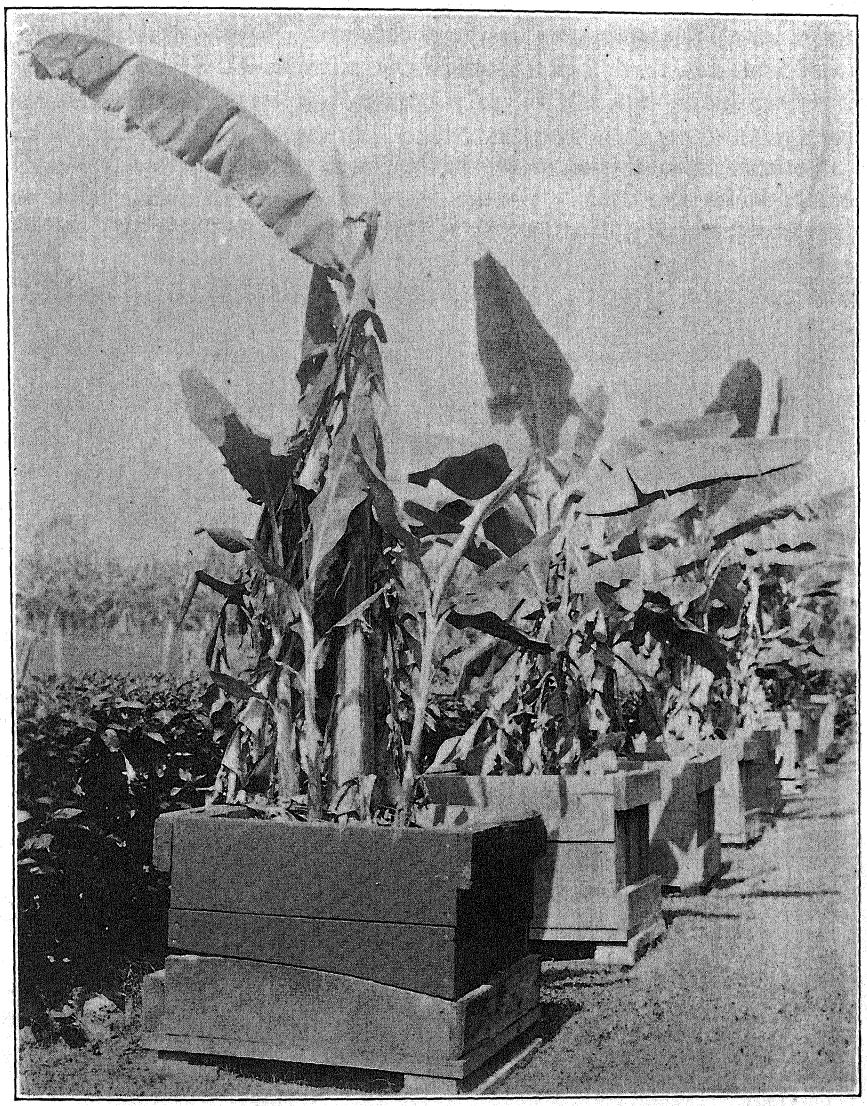

FIG. 2. One row of inoculation experimental plot II, using boxes of sterilized soil, above the ground, for planting. Banana wilt disease produced in Box No. 1 by inoculation with pure culture R 1, *F. cubense* isolated from vascular bundles in rhizome of wilt diseased banana plant. Note characteristic splitting of pseudostem and bending over of diseased leaves.

Sterilization of soil. The soil was sterilized by means of a special apparatus consisting of ¾-inch steam piping perforated with holes and so arranged that jets of steam from these perforations would reach all particles of soil. The apparatus was designed in the form of a large fork, with prongs of perforated tubing so designed that each tube was not over 6 inches from any portion of the soil in the entire tank. The forked apparatus was driven into the soil in the tanks and connected with a steam hose leading to a boiler. The top of the soil in the tanks was covered with a double layer of moistened, heavy burlap bags. For experimental Plot I, a steam boiler was used to provide the steam for sterilization. The soil in each tank was sterilized for two to three hours with live steam, derived

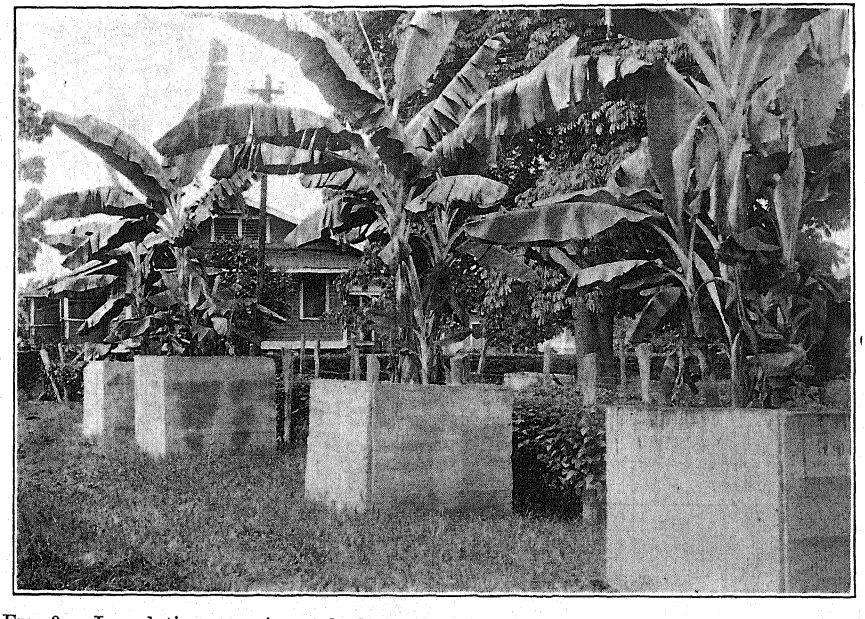

FIG. 3. Inoculation experimental plot III. Concrete tanks, with cement bases, above the ground. Four feet square inside.

at 110–120 pounds pressure in the boiler. The temperature maintained in the soil for this time was from 100° to 102° C. The soil in the wooden boxes of Plot II and the concrete tanks of Plot III were sterilized for two hours by live steam from a locomotive maintaining a pressure of 150 pounds; otherwise the operation was the same as in Plot I. The temperature of the soil in all parts of the boxes and tanks was 100–102° C. during the time of steaming. As soon as each tank or box of soil was sterilized, the sterilizing apparatus was removed, under sterile conditions, and a piece of heavy, sterilized, tarred paper, painted on both sides with a full strength solution

of carbolineum, was tightly fitted over the top of the soil. The soil in this way remained hot for two or three days after the time of sterilization. Tangle-foot was smeared over the top of each tank so as to prevent crawling insects from entering (Fig. 1). Each tank was planted with a banana plant, and the soil was inoculated as soon as it cooled off. Bacteriological and fungous tests made of the soil at the time of planting proved that the soil in every case was sterile except in Tank 4, Plot I, as shown in table 2.

It was found a little more difficult to sterilize the soil in the large $4\frac{1}{2} \times 9$ feet tanks, as only one half could be sterilized at one time with the apparatus at hand. Enough controls were maintained to prove the efficiency of the sterilization work.

"Bits" or rhizomes used for planting. The "bits" or portions of rhizomes used for planting were obtained from a district where the banana wilt disease was not found. These "bits" were carefully washed free from all dirt, trimmed up as much as possible with a sterilized machete so as to have clean-cut surfaces, and then sterilized for five minutes in a 2 to 1000 strength solution of mercuric chloride. They then were washed in sterile water and trimmed up again with a sterilized machete so as to remove as much as possible of the outer parts that may still have contained some of the poison. The "bits" were then immediately planted and the soil inoculated. Three "bits" were planted in the large concrete tanks in Plot I, and one "bit" in each of the other tanks and boxes.

Method of planting and inoculation. In planting, a square hole large enough to allow for the passage of the "bit" was cut into the center of the tar paper covering the soil in each tank. Under aseptic conditions a hole was dug in the soil, large enough for proper planting, and the "bit" inserted according to the general farm practice. Before covering with earth, the fungous culture used in each case was applied about the "bit" and in the soil. Cultures of the various fungi used for inoculation were made in pint fruit jars, using about one-half pint of a mixture of corn meal and soil upon which to grow the fungus. Other cultures were grown upon sterilized green bean pods in Erlenmeyer flasks, and in certain cases these were also applied. The cultures were from 8 to 15 days old at the time of application. They were applied by being poured over the "bits" and mixed with the soil about them. After the "bits" were entirely covered with soil, the flap of tarred paper was again dropped back over the soil, thereby completely covering the tank and preventing air-borne spores from entering the soil. This method gave the fungus used for inoculation an opportunity to spread and develop in a pure state throughout the soil about the "bit." As soon as suckers forced their way through the soil, the flaps of paper were thrown back to allow the plant to grow. All possible precautions were taken against contamination from outside sources. The plants thenceforward were allowed to care for themselves.

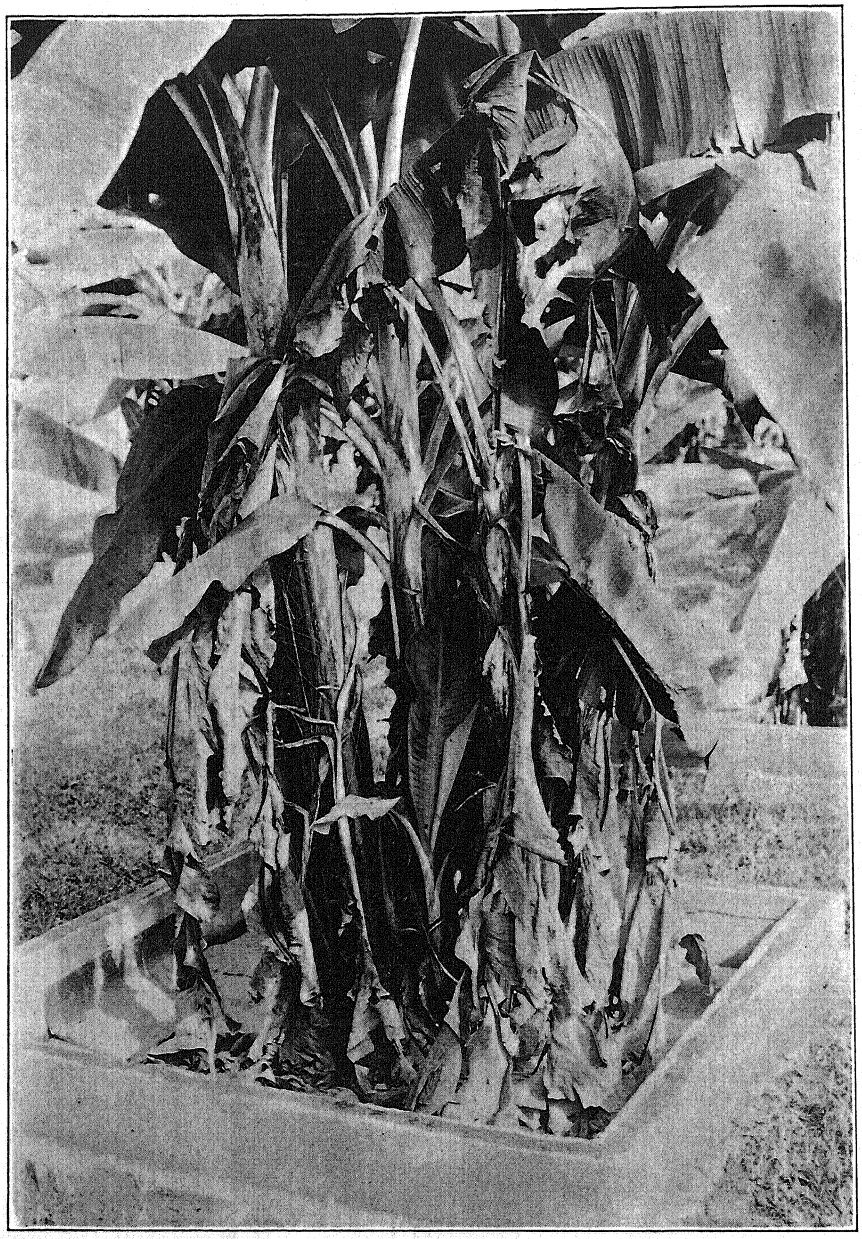

FIG. 4. Banana wilt disease produced by inoculation with pure culture R 17, *F. cubense* isolated from vascular bundles in rhizome of wilt diseased banana plant. Note characteristic wilting and drying up of leaves on young sucker in front. Tank No. 8.

CULTURES USED FOR INOCULATION

The cultures used for inoculation, as given below, were strains of the banana wilt organism (*F. cubense*); closely related organisms, isolated from the soil and plant parts, more or less difficult to distinguish from the wilt producing organism (*F. oxysporum* var. *nicotianae, F. orthoceras,* and *F. orthoceras* var. *triseptatum*); two organisms commonly associated with banana plants and frequently found about Colorado diseased plants (*F. moniliforme* var. *subglutinans,* and *F. moniliforme* var. *erumpens*); and two organisms, one commonly found on decaying banana trash (*F. anthophilum*) and the other in the soil (*Hypomyces ipomoeae*).

LIST OF CULTURES USED FOR INOCULATION

R 1. *Fusarium cubense.*

Isolated from vascular bundles in rhizome of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

R 3. *F. cubense.*

Isolated from vascular bundles in pseudostem of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

R 15. *F. cubense.*

Isolated from exterior of cut surface of pseudostem of wilt diseased Gros Michel banana (*Musa sapientum*). Truxillo, Honduras. Isolated Feb. 21, 1923.

R 17. *F. cubense.*

Isolated from vascular bundles in rhizome of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated April 15, 1923.

R 52. *F. cubense.*

Isolated from dead floral parts at tip of non-diseased banana fruit (*Musa sapientum*). Tela, Honduras. Isolated Jan. 12, 1923.

R 119. *F. oxysporum* var. *nicotianae.*

Isolation. Soil 1. Top soil under diseased bananas. Tela, Honduras. Isolated Nov. 17, 1922.

R 139. *F. oxysporum* var. *nicotianae.*

Isolation. Soil 35. Top soil under healthy bananas. Tela, Honduras. Isolated Jan. 15, 1923.

R 142. *F. oxysporum* var. *nicotianae.*

Isolation. Soil 38. Top soil under healthy bananas. Tela, Honduras. Isolated Jan. 15, 1923.

R 132. *F. orthoceras.*

Isolation. Soil 31. Top soil under healthy bananas. Tela, Honduras. Isolated Jan. 12. 1923.

R 45. *F. orthoceras* var. *triseptatum.*

Isolated from decaying old bunch of bananas (*Musa sapientum*) on ground. Tela, Honduras. Isolated Jan. 16, 1923.

R 182. *F. orthoceras* var. *triseptatum.*

Isolation. Soil 103. Top soil under diseased banana. Truxillo, Honduras. Feb. 23, 1923.

R 32. *F. moniliforme* var. *subglutinans.*

Isolated from decaying leaves of Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 17, 1922.

R 40. *F. moniliforme* var. *subglutinans.*

Isolated from vascular bundles in pseudostem of combination Colorado and wilt diseased banana plant (*Musa sapientum*). Isolation made Feb. 9, 1923, one day after cutting plant. Tela, Honduras.

R 58. *F. moniliforme* var. *subglutinans.*

Isolated from vascular bundles of pseudostem of Colorado diseased banana plant (*Musa sapientum*). Isolation made Feb. 9, 1923, one day after cutting plant. Tela, Honduras.

R 60. *F. moniliforme* var. *subglutinans.*

Isolated from vascular bundles of pseudostem of Colorado diseased banana plant (*Musa sapientum*). Isolation made Feb. 9, 1923, one day after cutting plant. Tela, Honduras.

R 62. *F. moniliforme* var. *crumpens.*

Isolated from vascular bundles of pseudostem of Colorado diseased banana plant (*Musa sapientum*). Isolation made Mar. 10, 1923, one day after collection. Tela, Honduras.

R 37. *F. anthophilum.*

Isolated from decaying banana trash (*Musa sapientum*). Tela, Honduras. Isolated Nov. 9, 1922.

R 184. *Hypomyces ipomoeae.*

Isolation. Soil 107. Top soil under healthy bananas. Truxillo, Honduras. Isolated Feb. 23, 1923.

PRELIMINARY INOCULATION EXPERIMENTS

R 2. *F. cubense.*

Isolated from vascular bundles in the rhizome of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

R 3. *F. cubense.*

Isolated from vascular bundles in pseudostem of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

R 11. *F. cubense.*

Isolated from vascular bundles in pseudostem of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

F. cubense isolated from wilt diseased plants was used primarily as a check on the other species. Brandes[5] has clearly shown that this organism is the cause of banana wilt. A very slight morphological and physiological difference was shown in some of these wilt-producing strains when not growing under normal conditions. Culture R 1 showed a tendency to run to mycelium and rarely produced spore bodies (sporodochia or pionnotes). *F. oxysporum* var. *nicotianae* isolated from the soil is very closely related to *F. cubense,* but does not produce the characteristic benzolic odor on rice

[5] Brandes, E. W. *Ibid.*

and has slightly larger spores and always a more abundant sclerotial formation. It is prevalent in the soil of diseased and non-diseased plantations. This resemblance made it necessary to test the pathogenicity of the fungus and in this way more clearly distinguish it, if possible, from *F. cubense*. *F. orthoceras* and *F. orthoceras* var. *triseptatum* are also commonly found in the soil and on decaying banana trash, and in some cases might be confused with a degenerate strain of *F. cubense*. Their relationship to *F. cubense*, therefore, also had to be tested out by the inoculation method.

FIG. 5. Banana wilt disease produced by inoculation with pure culture R 52, *F. cubense*, isolated from dead floral parts at tip of non-diseased banana fruit. Note splitting of pseudostem and characteristic bending over of diseased leaves. Box No. 9.

Another organism, *F. lutulatum,* is also closely related to *F. cubense.* This organism was not isolated until after the inoculation experiments were well under way. If future inoculation studies are undertaken, this species should be carefully tested. *F. moniliforme* var. *subglutinans* and *F. moniliforme* var. *erumpens* are distinctly different from *F. cubense* and could never be confused with the latter. They are commonly associated with banana culture and have been isolated from the vascular tissues of old Colorado diseased plants. Because of this relationship, these organisms were selected for inoculation purposes. The last two organisms used show no close relationship with *F. cubense. F. anthophilum* is commonly found on decaying banana trash, and the Fusarial stage of *Hypomyces ipomocae* is quite common in the soil of infested and non-infested plantations. These species were used to determine their pathogenicity, and also to act as a check on the entire group.

INOCULATION EXPERIMENT. PLOT I.

Table 2 gives the details of the inoculation experiment (plot I) carried out in concrete tanks built into the ground (Fig. 1). The method of sterilization, planting, and inoculation followed were the same as discussed under methods.

All plantings and inoculations were made the latter part of June and the first part of July, 1923, as indicated in table 2. Final results in most cases were not obtained until April 26, 1924, some 10 months from the time of inoculation. At this time, fruits, just below the English grade for maturity, were produced in tanks 2, 11, 12, 13, 15, 16, 17, and 22. The flower had "shot" in tanks 5, 18, and 21. All other plants either produced no flower or fruit or were diseased. The production of fruit and the healthy appearance of the plants showed that they were growing under good conditions for fruit production.

All of the cultures were of *Fusarium cubense,* and all except one (table 1) were obtained from banana wilt disease plants. One culture, R 52, was isolated from the dead floral parts at the tip of a banana finger of a bunch piled in a car at a fruit spur. Whether the organism was present in the car or developed in the field of course could not be definitely determined. It, however, might be safe to assume, because of this evidence, that *F. cubense* may be present to a certain extent along the railroad right-of-way through a non-diseased district. From these inoculations, positive cases of infection were obtained in tanks 1, 3, 6, 8, and 23. No disease was produced in tank 7 inoculated with culture R 3, *F. cubense.* This same culture produced disease in tank 1. The reason for using culture number R 3 in duplicate was due to the fact that, while it was a typical culture of *F. cubense* from a physiological and morphological standpoint, it did not

TABLE 2.—*Results of inoculating with Fusarium banana plants growing in concrete tanks sunk into the ground*[f]

Tank No.	Culture used	Planted and inoculated	Type of soil	Cut down	Wilt disease
		1923		1924	
1	R 3. *F. cubense*	June 16[a]	Light Sandy	Mar. 29	X[e]
2	R 119. *F. oxysporum* var. *nicotianae*	do[a]	do	do	—
3	R 15. *F. cubense*	do[a]	do	Oct. 8 (1923) and Mar. 13	X
4	R 58. *F. moniliforme* var. *subglutinans*	do[a]	do	Mar. 13	X[b]
5	Control, sterilized soil	do[c]	do	Apr. 26	—
6	R 1. *F. cubense*	do[a]	do	Mar. 13	X
7	R 3. *F. cubense*	do[a]	Fine Silt	Apr. 26	—
8	R 17. *F. cubense*	do[a]	do	Mar. 13	X
9	R 37. *F. anthophilum*	June 19[a]	do	Apr. 26	—
10	Control, sterilized soil	do[c]	do	do	—
11	R 132. *F. orthoceras*	July 5	Light Sandy	do	—
12	R 184. *Hypomyces ipomoeae*	do	do	do	—
13	R 62. *F. moniliforme* var. *erumpens*	do	do	Apr. 12	—
14	Control, unsterilized soil	do[d]	do	Mar. 13	X
15	R 139. *F. oxysporum* var. *nicotianae*	do	do	Apr. 26	—
16	R 60. *F. moniliforme* var *subglutinans*	do	do	do	—
17	Control, sterilized soil	do[c]	do	do	—
18	R 182. *F. orthoceras* var. *triseptatum*	do	do	do	—
19	R 40. *F. moniliforme* var. *subglutinans*	do	do	do	—
20	R 142. *F. oxysporum* var. *nicotianae*	do	do	do	—
21	R 45. *F. orthoceras* var. *triseptatum*	do[a]	do	do	—
22	R 32. *F. moniliforme* var. *subglutinans*	do	do	Apr. 12	—
23	R 52. *F. cubense*	do	do	Mar. 13	X
24	Control, sterilized soil	do[a]	do	do	—

[a] Replanted and reinoculated Aug. 10, 1923.

[b] Soil not thoroughly sterilized as shown by test at time of inoculation.

[c] Not inoculated; "Bit" planted in sterilized soil.

[d] Not inoculated; "Bit" planted in unsterilized, light sandy infected soil.

[e] X = disease present; — = disease absent.

[f] Tanks 1 to 4 inclusive, 6 and 7, 11 to 13 inclusive, 15 and 16, and 18 to 23 inclusive were inoculated with cultures growing on a mixture of cornmeal and soil and on green bean pods. Tanks 8 and 9 were inoculated with cultures growing on a mixture of cornmeal and soil. Controls, in which only sterilized bits were planted in sterilized soil and no fungous culture used, were scattered throughout the plot as represented in tanks 5, 10, 17, and 24. The tanks provided suitable checks upon the remaining inoculated ones. They were also checks to show whether or not the sterilization of the soil had been complete and whether there was any possible opportunity of infection from air-borne spores, insect-carried organisms or any other outside source. Tank 14 contained infested light sandy soil that had not been sterilized. This tank acted as a check on the sterilization of the infected soil and was used also to prove whether or not the

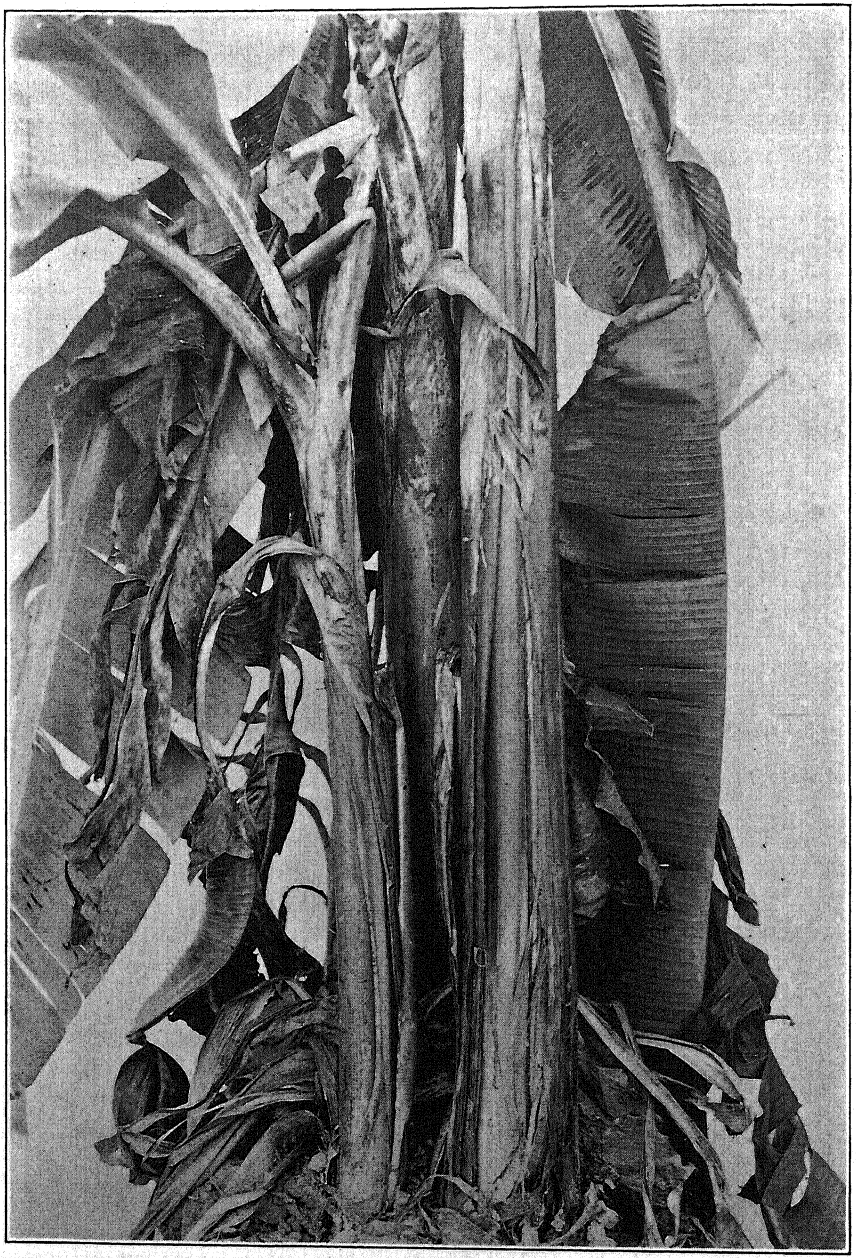

FIG. 6. Banana wilt disease produced by inoculation with pure culture R 2, *F. cubense*, isolated from the vascular bundles in the rhizome of a diseased banana plant. Note characteristic splitting of pseudostem. Preliminary inoculation experiment. Tank No. 1.

produce disease in the preliminary experiment as shown in table 1. Disease was also produced in tank 14, the control in which sterilized, infected, light sandy soil was used. This fact shows that the wilt organism occurs in the soil, and may be disseminated in soil. Disease was produced in one other case, in tank 4, inoculated with culture number R 58, *F. moniliforme* var. *subglutinans*. The disease was a typical case of banana wilt. Tests of the soil in this tank made at the time of inoculation showed the presence of Fusaria, indicating that the soil had not been thoroughly sterilized. The organism reisolated from the diseased plant was a pure culture of *F. cubense* and had no relation to *F. moniliforme* var. *subglutinans* used for inoculation. No significance, other than incomplete sterilization of soil, can be attached to this case of disease production.

The time of appearance of the disease after inoculation varied in the different tanks from two to seven and one-half months or longer. For example tank 1 was replanted and reinoculated on August 10, 1923. Disease was not recorded until March 29, 1924, just 7 months and 19 days after inoculation. Tank 3 was replanted and reinoculated on the same date. Disease appeared in one sucker on October 8, 1923, or two months after the time of inoculation. The disease did not appear in its virulent form until January 8, 1924, five months after inoculation.

The plants in all of the controls of sterilized and uninoculated soil, as represented by tanks 5, 10, 17, and 24, remained perfectly healthy. Tank 17 developed a bunch of fruit just below the English grade of maturity at the time of the close of the experiment on April 26, 1924.

No noticeable disease was produced by the inoculation of "bits" and soil with any of the other fungi used, including *F. oxysporum* var. *nicotianae, F. orthoceras, F. orthoceras* var. *triseptatum, F. moniliforme* var. *subglutinans, F. moniliforme* var. *erumpens, F. anthophilum,* and *Hypomyces ipomoeae*. In so far as these experiments are concerned, it was definitely proved that none of these cultures could produce disease either of the typical banana wilt type, or of the Colorado type. All the plants in these tanks were perfectly healthy and showed no evidence of root injury. It would not be safe to conclude that the first five of these fungi have no relation whatever to banana cultures. The possibility of a root infection being caused by some of this group has not been fully settled. The above experiments, however, are conclusive in regard to the direct role of these organisms in respect to the banana wilt and Colorado diseases.

In each case of disease production the original organism used for inoculation (*F. cubense*) was recovered in reisolations. The banana wilt organism was also definitely shown to be present with sporodochial production at the bend, on the exterior surface of diseased, bent-over leaves. Typical pure cultures of *F. cubense* were isolated from leaves of diseased

plants in tanks 3, 8, and 23. Positive isolations were also made from the leaves of the diseased plant in Box 9, Plot II. These results corroborate those obtained earlier by Brandes,[6] and show that the pathogene can be disseminated throughout the plantation by wind.

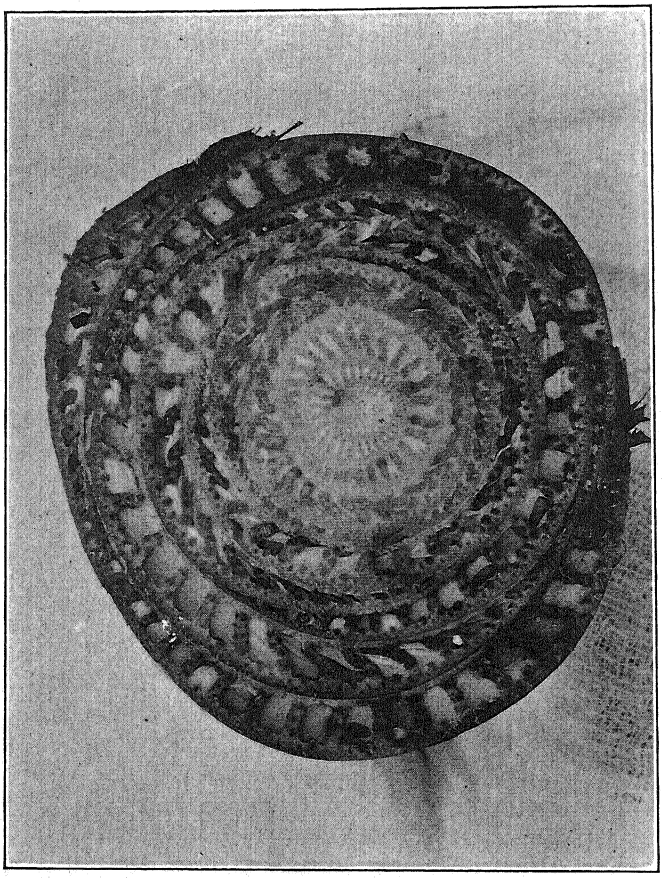

FIG. 7. Cross section of pseudostem of banana wilt diseased Gros Michel banana. Note discolored fibrovascular bundles more prevalent towards the exterior.

INOCULATION EXPERIMENT. PLOT II.

In order to have a check on the experiment carried out in Plot I, experimental Plot II was prepared with wooden boxes placed on the ground (Fig. 2). The purpose of the experiment was to provide a more careful control against possible outside sources of infection by raising the boxes off the ground, and also to make it possible to check up further the results obtained

[6] Brandes, E. W. *Ibid.*

by the cultures in Plot I. The details of the experiment are given in table 4. The arrangement, in so far as control and inoculation boxes are concerned, is similar to that discussed under Plot I.

TABLE 3.—*Results of inoculating with Fusarium banana plants growing in wooden boxes above ground*

Box No.	Culture used	Planted and inoculated	Type of soil	Cut down	Wilt disease
		1923		1924	
1	R 1. *F. cubense*	July 24	Fine Silt	Mar. 13	X
2	R 142. *F. oxysporum* var. *nicotianae*	do	do	Apr. 26	—
3	R 45. *F. orthoceras* var. *triseptatum*	July 18	do	do	—
4	R 139. *F. oxysporum* var. *nicotianae*	do	do	do	—
5	Control	do[a]	Light Sandy	do	—
6	R 60. *F. moniliforme* var. *subglutinans*	Aug. 10 July 18	do	do	—
7	R 182. *F. orthoceras* var. *triseptatum*	July 13	do	do	—
8	R 40. *F. moniliforme* var. *subglutinans*	do	do	do	
9	R 52. *F. cubense*	July 12	do	Mar. 13	X
10	R 45. *F. orthoceras* var. *triseptatum*	do	do	Apr. 26	—
11	R 15. *F. cubense*	do	do	do	—
12	Control	do[a]	do	do	—
13	R 3. *F. cubense*	July 6	do	do	—
14	R 119. *F. oxysporum* var. *nicotianae*	do	do	do	—
15	R 132. *F. orthoceras*	do	do	do	—
16	R 139. *F. oxysporum* var. *nicotianae*	do	do	do	—

[a] Not inoculated; "Bit" planted in sterilized soil.

As shown in table 3, the same cultures and soil used in Plot I were used in Plot II. The plantings and inoculations were made later, between July 6 and 18, 1923. The experiment was concluded April 26, 1924, or some 9 to 10 months later. Four different cultures of *F. cubense* were used, and, in two cases, in Boxes 1 and 9, typical wilt was produced. The interesting fact to note here is that the disease in these cases appeared approximately in the same time as it did when corresponding cultures were used for inoculation in Plot I, thereby checking up nicely on the pathogenicity of the cultures in question. Culture R 3 and R 15 failed to produce disease, although both were typical wilt-producing organisms and both had produced disease in Plot I. Box number 1 was inoculated with culture R 1 on July

24, 1923. On November 12, 1923, just 3 months and 19 days later, a decided splitting of the stem was observed. This splitting, and other symptoms of the disease, gradually became more pronounced (Plate 2). Box 9 was inoculated with culture R 52 on July 12, 1923. The first signs of the

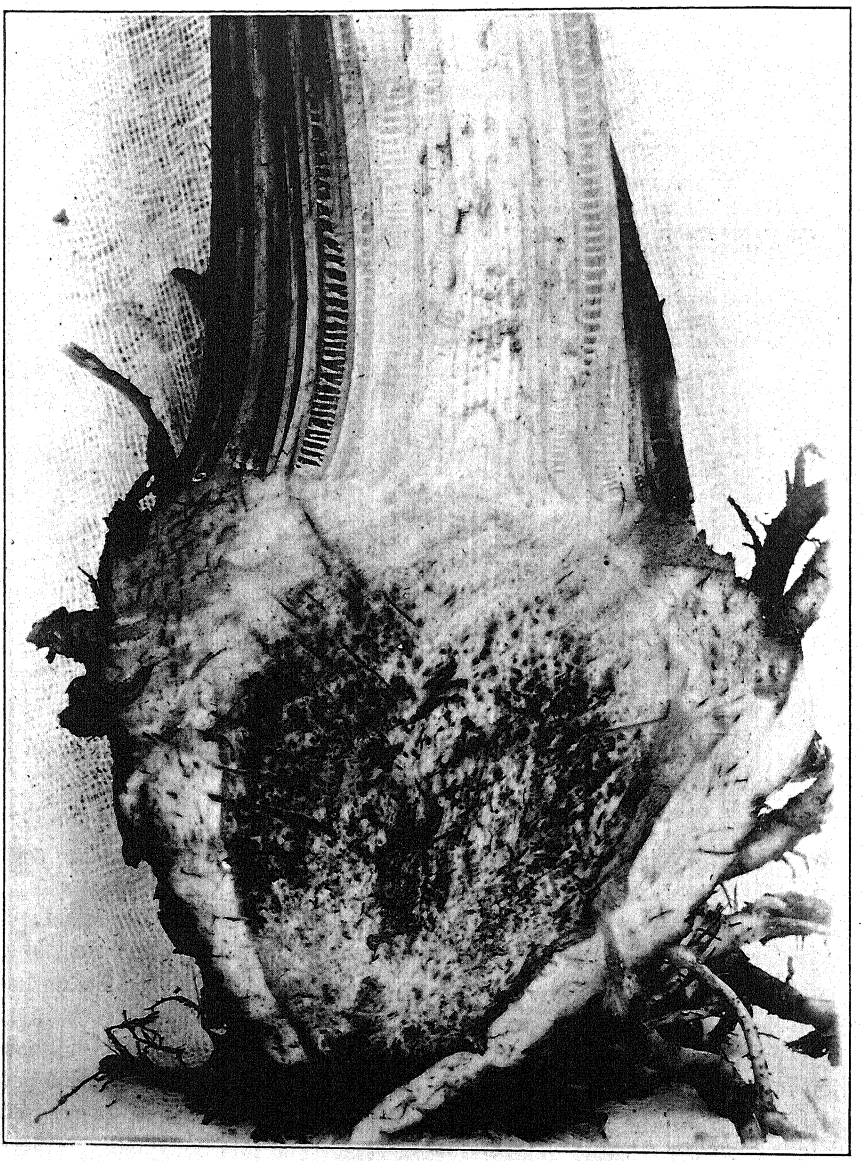

FIG. 8. Longitudinal section of banana wilt diseased Gros Michel banana. Note discolored fibrovascular bundles in rhizome and running into pseudostem.

typical disease were noted on December 5, 1923, not quite 6 months from the time of inoculation (Fig. 5). In no case was disease of any description produced in the other boxes inoculated with cultures coresponding to those used in Plot I, thereby verifying the results in the latter plot. The controls in every case remained perfectly healthy. Reisolations of the typical wilt organism similar to that used for inoculation were made from the two cases in which disease was produced.

INOCULATION EXPERIMENT. PLOT III

Experimental Plot III (Fig. 3) was a still further check on four cultures used in the main experiment, Plot I. The concrete tanks used in this experiment, being placed on the ground, were less subject to outside sources of infection. In the four tanks, representatives of the same cultures used in Plots I and II were used for inoculation, as shown in table 4. Inoculations were made on July 26, 1923, and the experiment was terminated on April 26, 1924.

TABLE 4.—*Results of inoculating with Fusarium banana plants growing in concrete tanks above ground*

Tank No.	Culture used	Planted and inoculated	Type of soil	Cut down	Wilt disease
		1923		1924	
1	R 119. *F. oxysporum* var. *nicotianae*	July 26	Fine Silt	Apr. 26	—
2	R 132. *F. orthoceras*	do	Light Sandy	do	—
3	R 139. *F. oxysporum* var. *nicotianae*	do	do	do	—
4	R 3. *F. cubense*	do[a]	do	do	—

[a] Reinoculated Nov. 1, 1923.

As indicated in table 4, only one culture of *F. cubense* (R. 3) was used for inoculation. No positive results were obtained with this culture. The failure of R 3 to produce disease in a number of the inoculations in Plots I, II, and III indicates that it is not extremely pathogenic under all conditions, although disease was produced by it in one instance (Plot I). The other cultures, R 119, *F. oxysporum* var. *nicotianae;* R 139, *F. oxysporum* var *nicotianae;* and R 132, *F. orthoceras,* again failed to produce disease of any kind, thereby verifying the results obtained in Plots I and II. Tank 3 at the close of the experiment had a bunch of fruit somewhat below the English grade in maturity. All of the plants in the experiment were perfectly healthy and normal.

DISCUSSION OF RESULTS

The results obtained in these experiments show conclusively that, of the organisms tested, only one distinct species, *F. cubense,* can produce the banana wilt disease. Isolation studies have shown that this one species only was found in all typical cases of banana wilt. Rarely have other Fusaria been found in the vascular bundles of diseased plants; and in these exceptional cases they have been isolated from plants with a complication of diseases, or after the portion of the plant from which the isolation was made had stood for a few days. It appears safe to say that *F. cubense* only can produce the typical banana wilt disease (Figs. 7 and 8), and that this organism only is found in young, typical cases of infection.

The role of other closely related species of Fusaria, especially *F. oxysporum* var. *nicotianae, F. orthoceras,* and *F. orthoceras* var. *triseptatum,* in relation to banana wilt appears to be negligible, as none of these were able to produce the disease. All three of the above organisms easily may be confused with *F. cubense,* especially when the latter is not in its normal state of development. This may also be true in regard to *F. oxysporum* and *F. lutulatum,* two species not tested from a pathogenic standpoint. When, however, *F. cubense* is in its typical and normal growth condition, the differences between it and the closely related forms are apparent. A detailed account of these differences will be given in a report on Fusaria in relation to banana plantations. In certain cases it still may be necessary to carry out inoculation experiments to distinguish definitely between a possible non-parasitic form of *F. cubense* and the parasitic species. Still further studies with these species might well be undertaken in the future to determine whether any root troubles can be produced by them.

While no culture of *F. cubense* isolated from the soil was used for inoculation purposes, it can safely be stated that this organism is present in the soil. This was clearly shown in the isolation, morphological, physiological, and inoculation studies. In the inoculation experiments, typical banana wilt disease was produced by planting a sterilized "bit" in a tank of unsterilized soil infested with *F. cubense. F. cubense* was reisolated from the diseased plant which showed the typical banana wilt symptoms. All precautions observed in the other tests were carried out in this tank experiment except that the soil was not sterilized. This test in itself shows conclusively that the causal organism is present in the soil and that it can be carried about by transporting infested soil.

The fact that the inoculation, morphological, and physiological studies have shown that a difference exists between *F. cubense* and the closely related forms found in and about banana plantations has a direct bearing on the question of the presence of *F. cubense* in virgin soil or upon dead and living vegetation found on, or growing in, virgin soil. These studies,

correlated with those made on the cultures isolated from several sources in and about banana plantations, including virgin soil and plants found thereon, makes it safe to say that the parasitic form of *F. cubense* is not present in virgin soil or upon dead or living plant material on lands that are virgin in so far as the banana plant is concerned. The organism apparently is closely confined to the banana plant and the soil in infected banana plantations. Once it becomes established in the soil, it retains its virulence for many years. There of course remains a possibility that a wild saprophytic form of *F. cubense* may be present in virgin soils and may gradually adapt itself to the banana plant and eventually assume a distinct parasitic nature. It is generally believed that this process of change requires many years. From a practical standpoint, however, it is safe to conclude that the distinctly parasitic form of *F. cubense* is definitely associated with the banana plant and infested banana plantations, and that the control measures will have to be based upon this conclusion.

The role of *F. moniliforme* var. *subglutinans* and *F. moniliforme* var. *erumpens,* the first of which is commonly found associated with living and decaying banana plants, has been in part rather definitely decided. *F. moniliforme* var. *subglutinans,* especially has been found to be associated with Colorado diseased plants. In order to test its pathogenicity by invading the plant through the underground parts, the comparatively large number of inoculations with this species were made. According to the results of these experiments, the organism, when inoculated into the soil and placed around the "bits" planted in the soil, has no definite parasitic nature. No disease was produced under the condition of the experiment. The test shows rather conclusively that the organisms in question have no direct relation to the Colorado disease, which may therefore be regarded as a disease produced by abnormal and poor cultural conditions. The organisms are extremely interesting because of their prevalence in and about banana plantations and might well bear watching.

The two other organisms tested, *F. anthophilum* and the Fusarial stage of *Hypomyces ipomoeae,* were definitely proved to be distinctly saprophytic in so far as the banana is concerned. The first species is very common on decaying banana trash, and the second is found on plants and in the soil of banana plantations and the forest. These organisms are not closely related to *F. cubense.*

SUMMARY

Only one distinct organism, *F. cubense,* is directly associated with typical cases of banana wilt and can produce the typical banana wilt disease.

Closely related species of Fusaria, especially *F. oxysporum* var. *nicotianae, F. orthoceras,* and *F. orthoceras* var. *triseptatum,* all of which are

found in diseased banana plantations, have no direct relation to banana wilt. The relation of the last two organisms to root troubles might well be studied further.

Fusarium cubense can be distinguished, in practically all cases, from closely related organisms. In certain doubtful cases, inoculation experiments will have to be resorted to in order to distinguish between a possible saprophytic form of *F. cubense* and the parasitic form.

Fusarium cubense is present in the soil, and from this source enters the plant and produces disease.

The inoculation studies correlated with the isolation, morphological, and physiological studies verify the statement made before, that *F. cubense* is not present in virgin soil in the parasitic form, which is closely associated with banana plants and the soil in infected banana plantations.

There is a possibility that a wild, saprophytic form of *F. cubense* is present in virgin soil and may gradually adapt itself to the banana plant and eventually assume a distinct parasitic nature. It is generally assumed that this process of change requires many years.

From a practical standpoint it is evident that the control work must be based upon the finding that the distinctly parasitic form of *F. cubense* is definitely associated with the banana plant and infested soil in banana plantations.

Fusarium moniliforme var. *subglutinans* and *F. moniliforme* var. *erumpens,* both quite generally found associated with living and decaying banana plants and often with the Colorado disease, have no direct relation to the Colorado disease. Because of the prevalence of these organisms in banana plantations, they might well bear watching.

Fusarium anthophilum, commonly associated with decaying banana trash, and the Fusarial stage of *Hypomyces ipomoeae,* found in soil and on dead plants in banana plantations and forests, have no direct relation to banana diseases.

AGRICULTURAL RESEARCH DEPARTMENT,
UNITED FRUIT COMPANY,
BOSTON, MASS.

THE EFFECTIVENESS OF VARIOUS FUNGICIDES IN CONTROLLING THE COVERED SMUTS OF SMALL GRAINS

RESULTS OF THE COOPERATIVE CEREAL SEED TREATMENT PROJECT OF THE CROP PROTECTION INSTITUTE

COMPILED BY

E. B. LAMBERT, H. A. RODENHISER, H. H. FLOR[1]

INTRODUCTION

Until recently formaldehyde and copper sulphate were the standard fungicides in the United States for controlling the covered smuts of cereals. However, when these fungicides are applied to wheat or hulless oats, they sometimes cause more loss through seed injury than they prevent by controlling smut. For this reason several modifications of the treatments have been devised in order to eliminate seed injury. Some of them are efficacious, but they usually make the treatment more complicated and cumbersome.

The attention of most investigators in this field was still directed to the modification of old treatments when Darnell-Smith (1), in 1917, devised the copper carbonate dust treatment for bunt of wheat. The announcement of this discovery soon diverted the attention of investigators in the United States from the modification of old methods to the thorough testing of the copper carbonate treatment and to the devising of new treatments. Preliminary tests with copper carbonate (5) were so satisfactory that the American Phytopathological Society in 1922 recommended extensive cooperative tests with this material. It was pointed out that the quickest method of determining the practical advantages and limitations of the dust treatment would be through the united efforts of many workers in widely separated regions, in order to determine its utility under many different soil and climatic conditions. The Advisory Board suggested that the Crop Protection Institute handle the project. At the Rochester meeting of the Institute, Dr. C. R. Orton and Dr. E. C. Stakman were appointed a committee to organize the project and secure cooperators.

The objects of the project, as outlined by the committee, were to determine the effect of chemical dusts on the amount of smut produced, and on

[1] The writers assisted Dr. C. R. Orton and Dr. E. C. Stakman, the committee on cereal seed treatments of the Crop Protection Institute, in drawing up the project. They also handled the details of supplying the cooperators with the necessary materials, and compiled the annual reports. They wish to express their appreciation to Dr. E. C. Stakman for his helpful suggestions and criticisms in the preparation of this manuscript.

the germination and yield of wheat, oats, and barley, and to compare the general effectiveness of the dusts with that of copper sulphate and of formaldehyde. In the spring of 1922 the project statement was distributed to all members of the Institute and to those pathologists who the committee thought would be interested in cooperating. Investigators in eleven states in the United States and four provinces in Canada made cooperative experiments in 1922, 1923, and 1924. Brief progress reports of these investigations were compiled annually (6, 7, and 8). Since the project was begun, many of the cooperators, as well as others, have published the complete results of their investigations. Their results are essentially in agreement with those obtained in the cooperative experiments. However, on account of the scope of the cooperative tests, it seems advisable to publish the results.

COOPERATORS

The following investigators cooperated in the project:

UNITED STATES

Dr. T. F. Manns,
Agricultural Experiment Station,
Newark, Del.

Dr. W. H. Martin,
New Jersey Experiment Station,
New Brunswick, N. J.

Dr. C. R. Orton,
Pennsylvania State College,
State College, Penn.

Dr. E. C. Stakman,
Minnesota Agricultural Experiment Station,
St. Paul, Minn., in cooperation with:

Mr. R. S. Dunham,
Northwest School and Station,
Crookston, Minn.

Mr. M. J. Thompson,
Northeast Experiment Station,
Duluth, Minn.

Mr. R. O. Bridgford,
West Central School and Station,
Morris, Minn.

Mr. R. E. Hodgson,
Southeast Experiment Station,
Waseca, Minn.

Dr. F. D. Heald,
State College of Washington,
Pullman, Wash.

Prof. Roy C. Thomas,
Agricultural Experiment Station,
Wooster, Ohio.

Prof. George H. Dugan,
Agricultural Experiment Station,
Urbana, Ill.

Prof. H. L. Bolley,
N. D. Agricultural Experiment Station,
Fargo, N. D.

Dr. R. C. Kirby,
N. Y. (Cornell) Agricultural Experiment Station,
Ithaca, N. Y.

Prof. A. T. Evans,
S. D. Agricultural Experiment Station,
Brookings, S. D.

Dr. C. W. Hungerford,
University of Idaho,
Moscow, Ida.

CANADA

Dr. B. T. Dickson,
MacDonald College,
MacDonald College, Que.

Prof. J. E. Howitt,
Ontario Agricultural College,
Guelph, Ont.

Dr. G. R. Bisby,
Manitoba Agricultural College,
Winnipeg, Manit.

Prof. W. P. Fraser,
University of Saskatchewan,
Saskatoon, Sask.

MATERIALS AND METHODS

There was some variation in the use of the different fungicides. It was originally suggested that the following fungicides be used: copper sulphate dip, formaldehyde spray, formaldehyde sprinkle, formaldehyde dip, copper carbonate dust, and dehydrated copper sulphate dust. However, many promising fungicides have been placed on the market since the project was begun in 1922. Several of these were included in the tests, and, in some instances, substituted for those listed above. Only three tests were made with the copper sulphate dip method; the results were inconclusive and have therefore been omitted from this paper. The following fungicides were used: formaldehyde dip, formaldehyde spray, copper sulphate plus lime dust, copper carbonate dust, nickel carbonate dust, Seed-O-San dust, Semesan dust, and solutions of Semesan and of Uspulun.

Many of the cooperators deviated slightly from the original plan of applying the fungicides. These variations apparently had little influence on the results obtained and will therefore not be discussed in detail. The dusts were usually applied at the rate of two ounces a bushel for wheat, and either two or three ounces a bushel for oats and barley. Formaldehyde was used in two strengths: as a dip or sprinkle diluted at the rate of one part of 40 per cent liquor of formaldehyde to 320 parts of water, followed by a one- to five-hour cover; or as a spray, diluted 1 : 1 with water, and applied at the rate of one quart of diluted solution to 50 bushels of grain, as recommended by Haskell (4). Semesan and Uspulun were applied in solution, in strengths of 0.30 per cent and 0.25 per cent, respectively, the period of soak varying from 15 minutes to two hours. Tests also were made with these fungicides after presoaking the grain.

In the original outline of the experiment it was suggested that triplicated one-fortieth acre plots be used. A provision was added that the best agronomic practice of the station at which the tests were made be followed, if it was necessary to deviate from this plan. It was evident from the reports of the cooperators that there had been but little uniformity either in size of plots or number of replications. The size of the plots varied from a single rod row to one-fortieth acre, and the number of replications from one to six. Because of these differences, it was impossible to analyze the data on a statistical basis.

TABLE 1.—*Results of treating wheat with various fungicides at 20 different stations in the United States and Canada in 1922, 1923, and 1924*

Station	Year	Treatments														
		Check			Formaldehyde dip (1–320)			Copper carbonate dust			Copper sulphate plus lime dust			Seed-O-San dust		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Alberta																
Lacombe	1922	100.0	16.9	25.3	90.0	0.0[d]	26.3	82.0	0.6	25.3	90.0	2.2	25.3	.....	.....	.....
	1923	68.0[c]	46.0	33.0	23.0[c]	0.0[d]	48.7	76.0[c]	1.0	51.0	.....	.....	.....	.....	.....	.....
Ontario																
Guelph	1923	91.0[c]	0.0	8.7	4.0[c]	0.0[d]	3.3	88.0[c]	0.0	6.9	86.0[c]	0.0	8.4	.....	.....	.....
Manitoba																
Winnipeg	1923	46.5	2.1	.....	37.0	0.0[d]	.....	41.5	Trace	.....	38.0	1.3	.....	36.0	1.0	.....
Brandon	1924	.....	22.7	.....	.....	1.0	.....	.....	7.3	.....	.....	.....	.....	.....	.....	.....
North Dakota																
Fargo	1923	.....	17.2	12.0[b, e]	.....	0.0[d]	11.2[b, e]	.....	0.0	11.8[b, e]	.....	0.0	11.8[b, e]	.....	0.0	12.3[b, e]
South Dakota																
Brookings	1922	.....	90.0	.....	.....	0.0[d]	.....	.....	0.0	.....	.....	.....	.....	.....	.....	.....
Idaho																
Moscow	1923	94.5	25.0	.....	.....	.....	.....	95.0	0.3	.....	86.0	2.6	.....	.....	.....	.....
	1924	99.0	16.2	52.7[b]	90.0	0.0	45.4[b]	95.5	0.2	58.1[b]	.....	.....	.....	.....	.....	.....
Washington																
Pullman	1923	.....	46.7	.....	.....	0.0[d]	.....	.....	5.9	.....	.....	.....	.....	.....	.....	.....
	1924	.....	36.2	.....	.....	0.0	.....	.....	0.0	.....	.....	.....	.....	.....	.....	.....
Ohio																
Wooster	1923	.....	46.0	.....	.....	.....	.....	.....	0.0	.....	.....	Trace	.....	.....	.....	.....
Illinois																
Urbana	1923	.....	0.0	4.9[b]	.....	0.0[d]	9.0[b]	.....	0.0	7.7[b]	.....	0.0	7.5[b]	.....	0.0	9.9[b]
Minnesota																
St. Paul	1922	.....	0.6	26.3[b]	.....	0.0[d]	20.9[b]	.....	0.0	28.5[b]	.....	.....	.....	.....	0.0	27.8[b]
	1923	87.0	24.3	20.5[b]	87.0	0.0[d]	21.8[b]	94.0	0.7	21.2[b]	.....	.....	.....	93.0	0.0	21.5[b]
	1924	80.0	15.5	28.3[b]	64.0	Trace	24.8[b]	82.0	Trace	34.0[b]	.....	.....	.....	89.0	2.0	34.3[b]
Crookston	1922	.....	11.3	20.6	.....	0.0[d]	17.4	.....	1.6	22.2	.....	.....	.....	.....	0.6	23.7
	1923	65.0	4.2	13.7	87.0	0.0[d]	9.6	94.0	0.0	13.2	.....	.....	.....	93.0	0.0	14.7
	1924	80.0	6.7	22.7[b]	64.0	0.0	25.6[b]	84.5	Trace	27.8[b]	.....	.....	.....	89.0	Trace	25.1[b]

TABLE 1.—(*Continued*)

Station	Year	Treatments													
		Check			Formaldehyde dip (1–320)			Copper carbonate dust			Copper sulphate plus lime dust			Seed-O-San dust		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota																
Grand Rapids	1922	.....	8.3	21.5	.....	0.0[d]	23.0	.....	0.3	22.3	.....	.....	.....	.....	1.0	22.3
Coon Creek	1924	80.0	13.0	.....	64.0	0.0	.....	84.5	0.0	.....	.....	.....	.....	89.0	Trace	.....
Morris	1922	.....	2.0	15.3	.....	0.0[d]	14.9	.....	0.0	18.2	.....	.....	.....	.....	2.6	17.4
	1923	65.0	2.0	10.8	87.0	0.0[d]	10.5	94.0	0.0	12.0	.....	.....	.....	93.0	0.0	11.6
	1924	80.0	10.7	22.6[b]	64.0	0.0	16.9[b]	84.5	Trace	22.3[b]	.....	.....	.....	89.0	Trace	21.2[b]
Waseca	1922	.....	6.5	22.5	.....	0.0[d]	18.8	.....	0.0	19.5	.....	.....	.....	.....	.....	.....
	1923	65.0	.....	16.3	87.0	.....	14.4	94.0	.....	16.1	.....	.....	.....	93.0	.....	14.1
Saskatchewan																
Indian Head	1922	90.0	5.5	53.7[e]	96.0	0.0[d]	53.3[e]	92.0	0.4	54.0[e]	98.0	0.7	58.7[e]	.....	.....	.....
(Exp. Farm)	1923	68.2	4.0	12.4	23.0	0.0[d]	13.5	76.0	0.0	17.2	75.0	0.0	16.2	.....	.....	.....
Indian Head (Dom. Lab.)	1922	90.0	15.8	.....	96.0	0.0[d]	.....	86.0	1.0	.....	94.0	3.1	.....	.....	.....	.....
Rosthern	1922	98.0	17.7	34.7[e]	94.0	0.0[d]	39.3[e]	100.0	1.3	39.3[e]	96.0	5.0	38.7[e]	.....	.....	.....
	1923	68.0	5.6	21.3	23.0	Trace[d]	21.3	76.0	Trace	22.3	.....	.....	.....	.....	.....	.....
Scott	1922	100.0	5.4	18.2	90.0	0.0[d]	16.3	82.0	0.0	17.3	90.0	Trace	16.6	.....	.....	.....
	1923	68.2	45.0	25.7	23.0	Trace[d]	45.5	76.0	0.7	44.1	75.0	0.5	46.7	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.

[b] Yield tests from rod rows.

[c] Germination in soil.

[d] Formaldehyde sprinkle.

[e] No replication.

The results reported by the various cooperators from 1921 to 1924, inclusive, are summarized in tables 1 to 8. Unless otherwise noted, the figures for yield are the averages for replicated plots, and the figures for percentage of germination are the results of tests made in sand.

RESULTS OF INVESTIGATIONS

Wheat. The data for wheat are given in tables 1 and 2.

The superiority of copper carbonate dust over the other fungicides tried is clearly indicated. It is apparent that, in nearly all of the tests, the various fungicides, with the exception of formaldehyde, had little or no effect on the germination of the seed. Numerous investigators reported apparent seed injury from the use of formaldehyde. At Guelph, Ontario, in 1923, germination was reduced from 99 per cent in the check to 4 per cent in the treated plot. Likewise, in the same year at Indian Head, Saskatchewan, formaldehyde apparently reduced germination from 68 per cent in the check to 23 per cent in the formaldehyde treated plot. Formaldehdye did not injure the seed at Indian Head, Saskatchewan, in 1922, nor at University Farm, St. Paul, Minnesota, in 1923. Some of the minor differences in germination may have been due to random sampling in poor seedlots.

Several of the fungicides effectively controlled bunt. Of these, formaldehyde was the most effective. At only one station did as much as 1 per cent of bunt develop in the formaldehyde plots. Copper carbonate dust controlled bunt satisfactorily in nearly all of the trials. It reduced the amount of bunt to less than 2 per cent, except in two trials. At Pullman, Washington, 5.9 per cent developed in 1923; and at Brandon, Manitoba, 7.33 per cent was recorded in 1924. In most of the tests, however, the bunt was almost entirely eliminated. The copper sulphate plus lime dust consistently reduced the amount of bunt but not sufficiently to justify its recommendation for practical use. Seed-O-San, nickel carbonate, and Semesan, applied as dusts, reduced the amount of smut, Semesan apparently being the most effective. Solutions of the organic mercury compounds, Semesan and Uspulun, were tested, but the results were somewhat conflicting. In 1924, Uspulun solution completely eliminated bunt of wheat at University Farm, Minnesota, and at Moscow, Idaho; but 1.83 per cent of bunt developed at Brandon, Manitoba. Semesan, when applied in solution, was effective in Minnesota and North Dakota; but 5.33 per cent and 2.07 per cent of bunt developed in treated plots at Brandon, Manitoba, and Moscow, Idaho, respectively.

Yield tests with wheat were inconclusive. There were twenty-four experiments in which yield comparisons were made between check plots and plots for seed treated with formaldehyde, and with copper carbonate dust. The plots from copper carbonate treated seed yielded more than the checks

TABLE 2.—*Results of treating wheat with various fungicides at 10 different stations in the United States and Canada in 1923 and 1924*

Station	Year	Treatments														
		Check			Nickel carbonate			Semesan soak 0.3 per cent			Semesan dust			Uspulun 0.25 per cent		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota																
St. Paul	1923	87.0	24.3	20.5[b]	92.0	2.3	23.3[b]	.....	.....	.....	.....	0.0	23.5[b]	.....	.....	.....
	1924	80.0	15.5	28.3[b]	76.5	4.0	33.5[b]	89.0	Trace	31.9[b]	86.5	Trace	26.7[b]	89.5	0.0	33.3[b]
Coon Creek	1924	80.0	13.0								86.5	Trace	.....	.....	.....	.....
Crookston	1924	80.0	6.7	22.7[b]	.....	.....	.....	.....	.....	.....	86.5	1.2	22.0	.....	.....	.....
Morris	1924	80.0	10.7	22.6[b]	.....	.....	.....	.....	.....	.....	86.5	Trace	21.9[b]	.....	.....	.....
Manitoba																
Winnipeg	1923	46.5	2.1	.....	44.5	3.5	.....	73.4	0.5	.....	.....	.....	.....	.....	.....	.....
Brandon	1924	.....	22.7	.....	.....	12.3	.....	.....	5.3	.....	.....	2.0	.....	.....	1.8	.....
Idaho																
Moscow	1923	94.5	25.0	.....	93.0	2.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	1924	99.0	16.2	52.7[b]	93.0	0.0	51.2[b]	96.0	2.1	52.7[b]	98.0	Trace	48.3[b]	100.0	0.0	55.6[b]
Washington																
Pullman	1923	.....	46.7	.....	.....	11.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	1924	.....	36.2	.....	.....	.....	.....	.....	.....	.....	.....	0.6	.....	.....	.....	.....
Ohio																
Wooster	1923	.....	46.0	.....	.....	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
North Dakota																
Fargo	1923	.....	17.2	12.1[b]	.....	0.0	13.0[b]	.....	0.0	12.0[b]	.....	0.0	13.5[b]	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.

[b] Yield tests from rod rows.

in seventeen of the twenty-four experiments and less than the checks in seven experiments. The formaldehyde plots yielded more than the check plots in nine experiments, less than the checks in fourteen experiments, and in one case the yield was identical with that of the check.

Hulled Oats. The data for hulled oats are given in tables 3 and 4.

None of the treatments seemed to have any consistent effect on seed germination of hulled oats: there was considerable fluctuation in the percentages of germination. In some cases treated seed did not germinate as well as untreated seed, while in others the reverse was true. It was impossible to determine from single tests whether these differences were due to random sampling or to the effect of the treatments. Experiments in which numerous check plots were planted proved this conclusively; for example, at Winnipeg in 1923 the average germination of untreated seed was 95 per cent, while the percentages for seed treated with formaldehyde and with copper carbonate were 84 and 66 respectively (table 3). These results might lead one to the conclusion that formaldehyde reduced germination 11 per cent, and that copper carbonate reduced it 29 per cent, but there were greater differences than this in the germination of replicated checks, and the apparent injury of treated seed was probably due to random sampling, as the percentage of germination of 16 random samples of untreated seed varied from 59 per cent to 100 per cent.

Formaldehyde was by far the most effective of the fungicides used for controlling smuts of oats, and was the only one which could be considered as entirely satisfactory. The other substances did not consistently reduce the percentage of smut. Seed-O-San markedly reduced the percentage of smut only at Fargo, North Dakota, in 1923. In three tests, at Winnipeg, Manitoba, in 1923, and at Wooster, Ohio, in both 1923 and 1924, seed treated with Seed-O-San produced more smut than did the untreated seed. Copper carbonate was the most effective of the dusts, although it did not control smut satisfactorily. In all cases it reduced the percentage of smut, but there was considerable difference in the extent to which it controlled the smuts in the experiments of different cooperators (table 3). Some of these differences in smut control may have been due to the fact that copper carbonate often is less effective on naturally inoculated seed than on that which has been artificially inoculated. Dr. R. S. Kirby emphasized this point in the conclusion of his 1923 report. He states: "Apparently the effectiveness of the various treatments can be determined only by the use of naturally inoculated seed. Artificial inoculation, when accomplished by mixing the seed with dry smut spores, does not allow the spores to get inside the glumes, or for them to germinate and form sporidia inside the glumes, as pointed out by Zade. This was shown in the 1923 experiments at Ithaca,

where the sulphur and copper carbonate dusts and Chlorophol steep treatments gave perfect control when artificially inoculated seed was used but gave only fair to moderate control when tested out on naturally inoculated seed."

Although nickel carbonate and copper sulphate plus lime dust reduced the smut in all trials, they usually were less effective than copper carbonate. The organic mercury compounds were unsatisfactory in practically all cases. Copper carbonate and nickel carbonate were the most promising dusts, while formaldehyde was distinctly better than solutions of Semesan and Uspulun.

Apparently none of the treatments consistently increased or decreased the yield. In most cases treated seed yielded more than the untreated, although the reverse was true in a few cases. This variation may have been due to soil heterogeneity. The only plots in which yields were determined, where the check plots were heavily smutted, were at St. Paul, Minnesota, in 1923. There the treated plots yielded considerably more than the checks and the difference in yield was roughly proportional to the degree of smut control.

Hulless Oats. The data for hulless oats are given in tables 5 and 6.

The tests with hulless oats were made at only a few stations—all in Canada—and fewer fungicides were used than for wheat and hulled oats. The results of germination tests, smut counts, and yield tests indicate that copper carbonate was the best of the fungicides tested. Formaldehyde was decidedly injurious to seed, while copper carbonate and copper sulphate plus lime dusts did not seem to affect seed germination. The dusts controlled the smut effectively in all of the tests. In two of them, formaldehyde reduced the percentage of smut only slightly and, in general, was not so effective as copper carbonate.

Nearly all of the treated plots yielded more than the checks. This was especially noticeable when the yields of copper carbonate plots were compared with those of badly smutted check plots. In some cases the treated plots yielded two or three times as much as the checks. For example, at Scott, Saskatchewan, in 1923, the check plot contained 62 per cent of smut and yielded 21.8 bushels an acre, while the copper carbonate plot was free from smut and yielded 52.8 bushels an acre. And at MacDonald College, Quebec, in 1922, the check plot contained 49 per cent of smut and yielded 12.5 bushels an acre, while the copper carbonate plots contained 0.3 per cent of smut and yielded 28 bushels an acre. Again, at MacDonald College, in 1923, the check contained 71.4 per cent of smut and yielded 12.3 bushels an acre, while the copper carbonate plot contained 1.5 per cent of smut and yielded 36.6 bushels an acre.

TABLE 3.—*Results of treating oats with various fungicides at 20 different stations in the United States and Canada in 1922, 1923, and 1924*

Station	Year	Treatments													
		Check			Formaldehyde dip (1–320)			Copper carbonate dust			Copper sulphate plus lime dust			Seed-O-San dust		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota																
St. Paul	1922	.....	1.0	55.5[b]	.....	0.0	48.4[b]	.....	0.0	51.1[b]	.....	.....	.....	.....	Trace	63.8[b]
	1923	83.0	38.7	47.6[b]	96.0	Trace	68.8[b]	92.0	6.0	60.0[b]	.....	.....	.....	98.0	19.6	57.0[b]
	1924	91.5	0.0	56.3[b]	79.0	0.0	62.1	91.5	0.0	58.9[b]	.....	.....	.....	84.0	0.0	39.0[b]
Crookston	1922	.....	Trace	77.0	.....	0.0	69.1	.....	Trace	81.3	.....	.....	.....	.....	Trace	72.6
	1923	83.0	10.1	49.9	96.0	0.0	48.4	92.0	7.6	53.9	.....	.....	.....	98.0	10.8	51.8
	1924	91.5	1.5	74.8[b]	79.0	0.0	76.8[b]	91.5	Trace	71.4[b]	.....	.....	.....	84.0	Trace	61.4[b]
Duluth	1922	.....	1.6	48.9	.....	Trace	38.5	.....	Trace	55.5	.....	.....	.....	.....	.....	.....
	1923	.....	3.7	56.8	.....	0.6	51.0	.....	0.8	55.6	.....	.....	.....	.....	2.3	55.0
Coon Creek	1924	91.5	7.0	.....	79.0	0.0	.....	91.5	5.0	.....	.....	.....	.....	84.0	6.3	.....
Waseca	1922	.....	2.5	55.1	.....	0.0	55.1	.....	Trace	52.0	.....	.....	.....	.....	.....	.....
	1923	.....	.....	46.2	.....	.....	42.3	.....	.....	48.9	.....	.....	.....	.....	.....	32.7
Morris	1924	91.5	5.3	71.8[b]	79.0	0.0	76.7[b]	91.5	2.3	68.4[b]	.....	.....	.....	84.0	3.3	72.9[b]
Saskatchewan																
Indian Head	1922	100.0	7.5	.....	94.0	0.0	.....	100.0	1.0	.....	100.0	5.6	.....	.....	.....	.....
	1923	.....	22.4	.....	.....	0.0	.....	.....	0.4	.....	.....	0.8	.....	.....	.....	.....
Ontario																
Guelph	1922	.....	2.2	76.0	.....	Trace[d] / 0.0[e]	76.1[d] / 74.6[e]	.....	1.4	77.3	.....	2.0	78.6	.....	.....	.....
	1923	98.0[c]	4.4	44.5	46.0[d] / 98.0[e]	Trace[d] / Trace[e]	36.9[d] / 46.0[e]	97.0[c]	1.2	44.8	94.0[c]	3.1	44.6	.....	.....	.....
	1924	95.0	1.3	.....	82.0[d] / 95.0[e]	0.0[d] / 0.0[e]	.....	.....	.....	.....	.....	.....	.....	93.0	0.3	.....
Quebec																
MacDonald College	1922	94.0	1.5	61.8[g]	.....	.....	.....	91.0	0.0	63.2[g]	.....	.....	.....	.....	.....	.....
Manitoba																
Winnipeg	1923	95.0	5.8	18.8[b]	84.0[d] / 84.0[e]	0.3[d] / 0.0[e]	21.4[d, b] / 18.1[e, b]	66.0	0.0	19.4[b]	81.5	Trace	20.0[b]	95.5	8.2	18.2[b]
Brandon	1924	.....	13.1	.....	.....	0.4	.....	.....	1.3	.....	.....	.....	.....	.....	.....	.....

TABLE 3.—(*Continued*)

Station	Year	Treatments													
		Check			Formaldehyde dip (1–320)			Copper carbonate dust			Copper sulphate plus lime dust			Seed-O-San dust		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ	Pct. smut	Acre[a] yields
North Dakota																
Fargo	1923	.....	5.1	45.2[b]	.....	0.0[d] / 0.0[e]	46.9[d, b] / 4.97[e, b]	.....	3.0	42.2[b]	.....	0.0	31.4[b]	.....	0.0	47.3[b]
Illinois																
Urbana	1922	.....	2.2	.....	.....	0.0	.....	.....	Trace	.....	.....	.....	.....	.....	.....	.....
	1923	.....	4.0	9.9[b]	.....	0.0[d] / 0.0[e]	12.5[d, b] / 11.1[e, b]	.....	1.8	11.5[b]	.....	1.9	11.5[b]	.....	3.8	12.7[b]
Ohio																
Wooster	1923	.....	2.1	.....	.....	0.8[d]	.....	.....	1.3	.....	.....	.....	.....	.....	3.6	.....
	1924	97.0[f]	35.6	.....	97.0[f]	0.0[d]	.....	96.0[f]	4.3	.....	.....	.....	.....	87.0	36.7[f]	.....
Pennsylvania																
State College	1922	.....	7.2	.....	.....	.....	.....	.....	3.6	.....	.....	.....	.....	.....	.....	.....
	1923	92.0	8.0	22.0[b]	85.0	0.0	.....	96.0	3.0	23.6[b]	.....	.....	.....	.....	.....	.....
	1924	88.0[f]	7.2	22.7[b]	88.0[f]	0.0	26.0[b]	78.0[f]	1.2	25.7[b]	.....	.....	.....	.....	.....	.....
New Jersey																
New Brunswick	1923	93.3	4.1	24.6[b]	96.3[d] / 96.3[e]	0.2[d] / 0.3[e]	25.1[d, b] / 25.9[e, b]	97.0	0.9	25.4[b]	96.6	1.2	22.9[b]	.....	.....	.....
	1924	100.0	4.1	35.1[b]	55.0	0.0	30.6[b]	98.0	0.4	36.0[b]	.....	.....	.....	.....	.....	.....
Delaware																
Newark	1922	.....	4.1	.....	.....	0.0[e]	.....	.....	Trace	.....	.....	2.5	.....	.....	.....	.....
	1923	.....	4.7	.....	.....	Trace	.....	.....	0.5	.....	.....	.....	.....	.....	.....	.....
Washington																
Pullman	1923	.....	30.0	73.0	.....	1.5	71.6	.....	1.0	79.1	.....	.....	.....	.....	.....	.....
Idaho																
Moscow	1924	100.0	16.0	.....	99.0	Trace	.....	100.0	1.8	.....	.....	.....	.....	.....	.....	.....
New York																
Ithaca	1923	.....	8.5	.....	.....	0.4[e]	.....	.....	1.5	.....	.....	.....	.....	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.
[b] Yield tests from rod rows.
[c] Germination in soil.
[d] Formaldehyde sprinkle.
[e] Formaldehyde spray.
[f] Germination on blotter.
[g] Yield tests on one-hundredth acre plots. No replications.

TABLE 4.—*Results of treating hulled oats with various fungicides at 14 different stations in the United States and Canada in 1922, 1923 and 1924*

Station	Year	Treatments													
		Check			Nickel carbonate			Semesan dust			Semesan soak 0.3 per cent			Uspulun 0.25 per cent		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota															
St. Paul	1923	83.0	38.7	47.6[b]	94.0	9.7	49.3[b]	89.0	17.7	63.2[b]	.....	.....	.....	.....	.....	.....
	1924	91.5	0.0	56.3[b]	90.5	0.0	53.1[b]	91.5	0.0	54.2[b]	96.5	0.0	55.2[b]	96.5	0.0	55.9[b]
Coon Creek	1924	91.5	7.0	.....	.....	.....	.....	91.5	5.7	.....	.....	.....	.....	.....	.....	.....
Crookston	1924	91.5	1.5	74.8[b]	.....	.....	.....	91.5	Trace	58.8[b]	.....	.....	.....	.....	.....	.....
Morris	1924	91.5	5.3	71.8[b]	.....	.....	.....	91.5	1.7	71.5[b]	.....	.....	.....	.....	.....	.....
Manitoba															
Winnipeg	1923	95.0	5.8	18.8[b]	82.5	2.7	19.0[b]	.....	.....	.....	87.5	1.1	19.6[b]	.....	.....	.....
Brandon	1924	.....	13.1	.....	.....	0.2	.....	.....	4.2	.....	.....	5.7	.....	.....	2.0	.....
Ontario															
Guelph	1923	98.0[c]	4.4	44.5	95.0[c]	2.4	40.2	.....	.....	.....	.....	.....	.....	.....	.....	.....
	1924	95.0	1.3	.....	93.0	Trace	.....	94.0	0.3	.....	91.0	Trace	.....	90.0	Trace	.....
North Dakota															
Fargo	1923	.....	5.1	45.2[b]	.....	0.0	35.7[b]	.....	0.0	45.9[b]	.....	0.0	45.0[b]	.....	.....	.....
Illinois															
Urbana	1923	.....	4.0	9.9[b]	.....	1.7	8.5[b]	.....	.....	.....	.....	.....	.....	.....	.....	.....
New Jersey															
New Brunswick	1923	93.3	4.1	24.6[b]	98.0	0.2	26.4[b]	98.3	3.8	25.7[b]	.....	.....	.....	.....	.....	.....
	1924	100.0	4.1	35.1[b]	100.0	0.2	35.9[b]	98.0	1.3	34.6[b]	100.0	0.4	36.8[b]	100.0	0.2	35.8[b]
Pennsylvania															
State College	1923	92.0	8.0	22.0[b]	94.0	0.5	25.6[b]	93.0	8.0	23.6[b]	.....	.....	.....	.....	.....	.....
	1924	88.0[d]	7.2	22.7[b]	92.0[d]	1.1	25.9[b]	90.0[d]	6.3	25.4[b]	88.0[d]	4.1	25.8[b]	.....	2.6	26.3[b]
Ohio															
Wooster	1923	.....	2.1	.....	.....	0.0	.....	.....	2.0	.....	.....	2.1	.....	.....	.....	.....
	1924	97.0[d]	35.6	.....	96.0[d]	3.6	.....	92.0[d]	23.5	.....	92.0[d]	12.2	.....	96.0[d]	6.3	.....
Idaho															
Moscow	1924	100.0	16.0	.....	99.0	9.4	.....	100.0	9.7	.....	100.0	9.1	.....	100.0	6.6	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.

[b] Yield tests from rod rows.

[c] Germination in soil.

[d] Germination in blotter.

TABLE 5.—*The results of treating hulless oats with various fungicides at seven stations in Canada in 1922, 1923 and 1924*

Station	Year	Treatments											
		Check			Formaldehyde dip (1–320)			Copper carbonate			Copper sulphate plus lime dust		
		Pct.[b] germ.	Pct. smut	Acre[a] yields	Pct.[b] germ.	Pct. smut	Acre[a] yields	Pct.[b] germ.	Pct. smut	Acre[a] yields	Pct.[b] germ.	Pct. smut	Acre[a] yields
Saskatchewan													
Indian Head	1923	71.5	20.1	25.4	33.5	13.8[c]	25.8	69.0	0.8	28.1	84.0	0.4	31.4
Scott	1923	57.0	62.5	21.8	17.5	1.6[c]	50.2	51.5	0.0	52.8	.....	.....	.....
Rosthern	1923	70.5	22.0	40.3	33.0	5.9[c]	38.9	70.5	Trace	44.6	.....	.....	.....
Alberta													
Lacombe	1923	59.5	35.3	41.3	16.5	1.9[c]	52.8	46.0	Trace	55.6	.....	.....	.....
Manitoba													
Brandon	1924	.....	52.1	.....	.....	14.9[c] / 42.3	.....	.....	3.3	.....	.....	.....	.....
Quebec													
MacDonald College	1922	82.0	49.0	12.5[e]	65.0[c] / 23.0[d]	1.0[c] / 0.3[d]	14.0[c, e] / 16.0[d, e]	90.0	0.3	28.0[e]	89.0	0.3	28.0[e]
	1923	93.0	71.4	12.3	77.0[c] / 0.7[d]	1.8[c]	33.5[c]	93.0	1.5	36.6	80.0	1.5	38.6
	1924	.....	58.4	.....	.....	3.3	.....	.....	2.1	.....	.....	.....	.....
Ontario													
Guelph	1922	.....	4.5	15.3[f]	.....	.....	.....	.....	0.2	15.7[f]	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.
[b] Germination in soil.
[c] Formaldehyde sprinkle.
[d] Formaldehyde spray.
[e] Yield tests on one-hundredth acre plots. No replications.
[f] No replications.

TABLE 6.—*Results of treating hulless oats with various fungicides at two stations in Canada in 1924*

Station	Year	Treatments													
		Check			Nickel carbonate			Semesan dust			Semesan soak 0.3 per cent			Uspulun 0.25 per cent		
		Pct. germ.	Pct. smut	Acre yields	Pct. germ.	Pct. smut	Acre yields	Pct. germ.	Pct. smut	Acre yields	Pct. germ.	Pct. smut	Acre yields	Pct. germ.	Pct. smut	Acre yields
Quebec																
MacDonald College	1924	.....	58.4	.....	.....	3.8	.....	.....	6.0	.....	.....	6.4	.....	.....	3.4	.....
Manitoba																
Brandon	1924	.....	52.1	.....	.....	3.4	.....	.....	14.3	.....	.....	16.4	.....	.....	11.3	.....

Barley. The data for barley are given in tables 7 and 8.

The tests with barley were not as extensive as those with either wheat or oats. In most cases they were not conclusive because of the small amount of smut in the checks. There seemed to be little or no seed injury as a result of any of the treatments. Many of the treatments reduced the amount of smut, but formaldehyde was the only fungicide that consistently controlled it. The yield tests also were inconclusive.

DISCUSSION AND CONCLUSIONS

The advantages of dusts are obvious. However, none of those tested controlled the smuts of hulled oats and barley as satisfactorily as did the liquids. It therefore seems necessary to recommend a liquid treatment for hulled grain.

Copper carbonate dust seems to be the best fungicide for wheat and hulless oats, whereas formaldehyde is most effective for hulled oats and barley. Copper carbonate controls bunt of wheat and the smuts of hulless oats cheaply and effectively without injuring the seed. In addition, it has the advantage of being applied as a dust. The formaldehyde and copper sulphate treatments control the smuts of these grains, but they wet the seed and often injure the seed.

Formaldehyde controlled the smuts of hulled oats more satisfactorily than any of the other materials tested. The formaldehyde spray method, recommended by Haskell (4), seems to be the most desirable for oats. This treatment is too injurious for use on wheat or hulless oats, but apparently it is safe for hulled oats and has the advantage of being practically a dry treatment, as the seed does not become wet if the formaldehyde is applied properly.

None of the dusts consistently controlled smuts of oats. Copper carbonate, which was the most effective of them, eliminated the smuts completely in some tests but not in others. Recent investigations by Zade (9) suggest plausible explanations for the disagreement in the results obtained by different cooperators. He found that *Ustilago avenae* persists from one crop year to the next in the form of mycelium and chlamydospores under the glumes and in the epidermis of the caryopsis. Thus the disparity in the results obtained by different investigators may have been due to the fact that some used naturally inoculated seed, while others used artificially inoculated seed. On the other hand, it is possible that copper carbonate dust controls the covered smut but not the loose smut, and that the difference in the effectiveness of the dusts in the different experiments was due to the amount of loose smut present in the seedlots.

TABLE 7.—*Results of treating barley with various fungicides at 10 stations in the United States and Canada in 1922, 1923, and 1924*

Station	Year	Treatments													
		Check			Formaldehyde dip (1–320)			Copper carbonate			Copper sulphate plus lime dust			Seed-O-San		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota																
St. Paul	1922	.....	0.0	61.2[b]	.....	0.0[c]	65.3[b]	.....	0.0	60.8[b]	.....	.....	.....	.....	0.0	60.0[b]
	1923	81.0	1.3	47.7[b]	76.0	0.0[c]	43.7[b]	83.0	1.0	49.6[b]	.....	.....	.....	87.0	0.8	42.4[b]
	1924	90.3	0.0	32.7[b]	88.0	0.0	31.8[b]	92.5	0.0	31.2[b]	.....	.....	.....	89.5	0.0	31.0[b]
Crookston	1922	.....	1.3	48.6	.....	0.0[c]	62.6	.....	0.6	62.3	.....	.....	.....	.....	0.6	46.8
	1923	72.5	3.5	31.4	76.0	Trace	40.3	83.0	0.7	40.7	.....	.....	.....	87.0	1.5	39.8
	1924	90.3	0.0	48.6[b]	88.0	0.0	53.1[b]	92.5	0.0	41.7[b]	.....	.....	.....	89.5	0.0	44.3[b]
Coon Creek	1924	90.3	3.3	20.5[b]	88.0	1.3	20.5[b]	92.5	0.8	22.2[b]	.....	.....	.....	89.5	2.7	23.8[b]
Morris	1922	.....	0.0	38.1	.....	0.0[c]	44.4	.....	0.0	41.2	.....	.....	.....	.....	0.0	43.0
	1923	72.5	5.3	27.9	76.0	Trace	27.4	83.0	4.0	29.3	.....	.....	.....	87.0	4.3	29.7
	1924	90.3	2.3	18.5[b]	88.0	Trace	17.8[b]	92.5	1.0	18.3[b]	.....	.....	.....	89.5	2.3	20.3[b]
Waseca	1923	72.5	.....	11.7	76.0	.....	10.7	8.3	.....	15.0	.....	.....	.....	87.0	.....	10.6
Saskatchewan																
Indian Head	1923	.....	6.6[d]	.....	.....	0.0[d, c]	.....	.....	2.0[d]	.....	.....	.....	.....	.....	.....	.....
Manitoba																
Brandon	1924	.....	7.9	.....	.....	3.8 / 2.7[c]	.....	.....	3.6	.....	.....	.....	.....	.....	.....	.....
North Dakota																
Fargo	1923	.....	Trace	35.9[b]	.....	0.0[c]	38.1[b]	.....	0.0	30.3[b]	.....	0.0	28.7[b]	.....	0.0	32.8[b]
Illinois																
Urbana	1923	.....	0.4	13.4[b]	.....	0.0[c]	9.3[b]	.....	0.0	12.3[b]	.....	0.7	15.3[b]	.....	0.0	12.5[b]
New York																
Livingston Co.	1923	.....	4.2	.....	.....	0.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.

[b] Yield tests from rod rows.

[c] Formaldehyde sprinkle.

[d] No replications.

TABLE 8.—*Results of treating barley with various fungicides at seven stations in the United States and Canada in 1923 and 1924*

Station	Year	Treatments													
		Check			Nickel carbonate			Semesan dust			Semesan soak 0.3 per cent			Uspulun 0.25 per cent		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota															
St. Paul	1923	81.0	1.3	47.7	79.0	2.2	46.4	87.0	1.0	53.3	.....	.....	.....	.....	.....	.....
	1924	90.3	0.0	32.7	96.5	0.0	32.4	90.5	0.0	34.3	93.0	0.0	33.8	91.5	0.0	32.4
Coon Creek	1924	90.3	3.3	20.5	.....	.....	.....	90.5	1.7	23.0	.....	.....	.....	.....	.....	.....
Morris	1924	90.3	2.3	18.5	.....	.....	.....	90.5	0.7	16.5	.....	.....	.....	.....	.....	.....
Crookston	1924	90.3	.....	48.6	.....	.....	.....	90.5	.....	48.3	.....	.....	.....	.....	.....	.....
North Dakota															
Fargo	1923	.....	Trace	35.9	.....	0.0	30.0	.....	Trace	43.8	.....	0.0	39.1	.....	.....	.....
Illinois															
Urbana	1923	.....	0.4	13.4	.....	0.4	17.5	.....	.....	.....	.....	.....	.....	.....	.....	.....
Manitoba															
Brandon	1924	.....	7.9	.....	.....	1.9	.....	.....	6.2	.....	.....	7.8	.....	.....	2.3	.....

[a] Yield tests from rod rows.

None of the tests with barley were conclusive because there was very little smut in the check plots. However, formaldehyde seemed to control the covered smut most effectively.

It is interesting to note that the most satisfactory treatments, the copper carbonate dust method and the formaldehyde spray method, both were devised in the past decade. New materials are being produced and offered to the experiment stations for trial, usually under trade names, and some of them may prove to be better than those now recommended. However, copper carbonate dust for wheat and hulless oats, and the formaldehyde spray for hulled oats are effective and simple treatments. If new fungicides replace these materials, it would seem that they must do so by their ability to increase yields distinctly, or to control seed- and soil-borne organisms in addition to the covered smuts.

The necessity of making comparative field tests of the value of different fungicides under many different soil conditions, and of carefully considering the effect of the fungicides on seed germination and on yields, cannot be over-emphasized. Laboratory indices of the effect of fungicides on pathogenes and on seed germination, as suggested by Gassner (2 and 3), may be used advantageously to show which chemicals deserve further test, but such tests must be supplemented by field experiments. Furthermore, clean seed is often treated, and therefore the relative effect of fungicides on yield of clean seed must be determined as well as their effect on the yields of badly smutted seed. Germination and yield tests must be made in such a manner as to make possible a statistical analysis of the results; otherwise they will be practically worthless.

SUMMARY

1. This paper summarizes the results of a cooperative project carried out in eleven states in the United States and four provinces in Canada during 1922, 1923, and 1924.

2. The primary object of the project was to compare the general effectiveness of the copper carbonate dust treatment with the standard formaldehyde treatment on the amount of smut and on seed germination and yield of wheat, oats, and barley. Several other materials were tested: nickel carbonate dust, copper sulphate plus lime dust, Semesan dust, and solutions of Uspulun and Semesan.

3. Copper carbonate dust was the most satisfactory of the materials tested for controlling bunt of wheat and smuts of hulless oats. It did not injure the seed appreciably, practically eliminated the smuts, and seemed to have a tendency to increase yields. Formaldehyde controlled the smuts of these cereals more completely than any of the other materials tested, but

often seemed to injure the seed. It was especially injurious to hulless oats. Solutions of Uspulun and Semesan were not as effective as formaldehyde.

4. Haskell's formaldehyde spray method seemed to be the best treatment for hulled oats. Formaldehyde controlled the smuts of hulled oats more completely than any of the other fungicides. Copper carbonate was the best of the dusts but was much less effective on hulled oats than formaldehyde.

5. The experiments with barley were somewhat inconclusive owing to the small percentages of smut in the check plots, but formaldehyde seemed to control the covered smut better than any of the other treatments. Apparently none of the treatments injured barley seed.

6. In most cases no definite conclusions could be drawn regarding the effect of the different treatments on yields, owing to the lack of systematic replication of the experimental plots. However, the results obtained from treating badly smutted hulless oats with copper carbonate dust were so consistent and outstanding that the treatment undoubtedly was at least partially responsible for the increased yield.

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STUDIES ON LEAFHOPPER INJURY TO APPLE LEAVES[1]

A. A. GRANOVSKY[2]

WITH PLATE XXIV

During the past three years, in many orchards at Sturgeon Bay and other points of the Peninsular region of Wisconsin, a peculiar yellowing of the marginal areas of apple leaves was a common occurrence. The symptoms in various degrees of intensity were observed on younger, as well as older, leaves, and could not be ascribed to any well known cause. This yellowing was often noted in association with curling of the leaves caused by aphids and leafhoppers commonly found on apple trees.

Observations in numerous orchards showed that the three species of the leaf-feeding apple aphids were present every year in abundance, infesting the branches, terminal shoots, fruit spurs and water sprouts. The green apple aphid, *Aphis pomi* De Geer, was found to feed largely on rapidly growing tips and water sprouts. The apple grain aphid, *Rhopalosiphum prunifoliae* (Fitch), formerly known as *Aphis avenae,* was confined to apple trees only in the spring and fall, spending its summer on grains and grasses. The rosy apple aphid, *Anuraphis roseus* Baker, commonly known in this country as *Aphis sorbi,* selects its feeding places on the leaves of terminal shoots and fruit spurs, usually on the lower part of the apple tree, until midsummer, when it migrates to the narrow-leaved plantain and returns to the apple trees in the fall.

It was further observed that the leafhoppers of cosmopolitan food habits were also present on apple trees in abundance. They were feeding not only on aphid infested branches, but were often found mixed on the same leaves with the aphids. The following species of leafhoppers were collected in such association: the apple leafhopper, *Empoasca maligna* (Walsh), formerly *E. unicolor* Gillette; the potato leafhopper, *Empoasca fabae* (Harris), commonly known as *E. mali* (Le Baron); a species known as *Empoasca flavescens* (Fabricius), which is closely related to the last named species in structure and in food habits; and the rose leafhopper, *Typhlocyba rosae* (Linnaeus), usually called *Empoa rosae* (Linn.). All the above listed species were observed feeding and breeding on the apple foliage.

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Occasionally the aster leafhopper, *Cicadula sexnotata* (Fallen), was collected. This species was always found in the mature stage, while other species were observed also in nymphal stages.

SYMPTOMS

The typical symptoms in question manifest themselves in a characteristic marginal yellow discoloration of the leaves. It usually begins at the tip of a leaf blade and gradually progresses downward along the midrib and lateral veins in more or less triangular areas at the outer margin of the leaf. This marginal discoloration, when viewed from the upper side of the leaf blade, is invariably more rapid on the proximal side of the lateral veinlets than on the distal side of these veins (Fig. 1). As the symptoms progress, the marginal yellow triangular areas running towards the midrib increase in size and occasionally may reach the midrib. In cases of severe injury, the tips, as well as the distal margins of the leaves, turn brown, showing a characteristic burning. In many instances leaf margins thus affected curled upward, much resembling the hopperburn injury of potatoes (Fig. 2). Although both older and younger leaves are subject to the symptoms, this is especially true of the younger leaves of rapidly growing terminal shoots and the succulent growth of water sprouts. A considerable pocketing and a characteristic downward rolling of the younger leaves was also observed, while the older leaves had only the discoloration symptoms described above without much downward rolling.

With the advent of cold weather the yellow discoloration at first assumed a reddish color which was very prominent, and later it gradually changed to a purple tinge. The normal and unaffected leaves remained green until the first killing frost. Not only were the affected triangular areas between the lateral veinlets changed in color by the approach of cold weather, but the veins and midribs of the affected leaves showed the purplish discoloration far beyond the farthest advance of the triangular discoloration, indicating the possible presence of some infective principle in the vascular system. The progressive downward discoloration along the veins suggests its presence in the phloem region. This, in its turn, suggested the possible insect relation to the symptoms described, as it is illustrated by Woods (19), Davidson (9), and Horsfall (15), that the sucking insects derive their food from the phloem vessels of the vascular bundles.

THE ASSOCIATION OF INSECTS WITH SYMPTOMS

Since these symptoms were found in the field on the leaves infested with the aphids and leafhoppers, these insects were suspected to have some causal

relationship. Yet it will not be amiss to mention that the symptoms are decidedly different from the feeding injury commonly ascribed to these insects. Several entomological workers reported a severe feeding injury to apple trees, especially to nursery stock, both by the leafhoppers and aphids. All of these insects retard the growth of the terminal shoots and nursery stock by extracting the plant juices.

So far as the leaf symptoms are concerned, Lathrop (16) and Ball (7) note that the apple leafhopper, *Empoasca maligna,* and the rose leafhopper, *Typhlocyba rosae,* produce only white stippling and spotting on the upper surface of the older leaves without curling them. The potato leafhopper, *Empoasca fabae,* is feeding on the younger leaves of tips, water sprouts and nursery stock, causing a characteristic downward curling and distortion of the foliage. The tips and margins of badly affected foliage turn brown and dry up without white spotting. Similar description of the feeding injury by *Empoasca fabae* is made by Ackerman (1) as well as others.

No reference to a triangular discoloration along the lateral veinlets and marginal upturn of the leaves is made, and, as far as the writer is aware, there is no definite record of these symptoms in literature on the subject.

In discussing the potato hopperburn, the apple plant is usually listed as a food of leafhoppers, and only a few brief notes [Ball (4, 5, 6), Parrott and Olmstead (18), Fenton and Hartzell (14) and others] mention the fact that leafhoppers may cause curling and tipburn. Only in two instances, Dudley and Wilson (10) and Dudley (11), we find that, in connection with potato hopperburn studies, when the potato leafhoppers were allowed to feed on a few apple seedlings under controlled conditions, they produced burning of the apple foliage in from 10 to 21 days. No description of characteristic symptoms of apple injury was given, however.

The literature on feeding habits of apple-inhabiting aphids also refers to curling of the leaves. The rosy apple aphid, *Anuraphis roseus,* is well known for its severe injury to apple trees by curling and twisting the leaves in clusters and often rendering fruits unmarketable (Baker and Turner (3), Matheson (17) and others). The green apple aphid, *Aphis pomi,* as reported by Matheson (17), is also able to contort the leaves, but the experiments conducted by Baker and Turner (2) indicate that this aphid causes very little leafcurling. The observations, as well as experiments, of several workers show that the apple grain aphid, *Rhopalosiphum prunifoliae,* seldom, if ever, causes any marked curling of the leaves.

Ackerman (1) differentiates the leafhopper curling of apple foliage from that of aphids. He states that the leafhopper curling begins "at the apex and extends toward the base of the leaves, the lower surface always being rolled in. This type of injury differs from aphid leaf-curl in that

aphids roll the leaves more tightly and curl them from the sides instead of from the tips.''

Again, in discussing the types of injury by aphids, no reference is made to characteristic discoloration symptoms and marginal upward curling of the leaves.

EXPERIMENTAL EVIDENCE OF THE DISEASE

In order to determine the cause of this injury, several series of cage experiments were conducted at the Peninsular Branch Experiment Station, Sturgeon Bay, Wisconsin. The first preliminary series of experiments was started on July 10, 1924, in the orchard at the station grounds, and closed on August 11, 1924. The second series of experiments was conducted between May 20 and August 13, 1925. In all experiments each spur under observation was covered with a large parchment bag of a type commonly used in plant breeding, which allowed enough light for normal development of leaves and at the same time protected them from insects and other external contaminating factors.

On July 10, 1924, ten rapidly growing terminal spurs without evidence of symptoms, and apparently healthy, on Wealthy apple trees were selected. Only ten upper leaves were left on each spur, the lower leaves being stripped off the shoot. Twenty leaf-hoppers of different instars were placed on the foliage of five of these spurs and inclosed by the large parchment bags.

TABLE 1.—*Relation of feeding of Empoasca fabae to apple leaf injury, 1924*

No. of spur	Spurs with leafhoppers			Total	No. of spur	Spurs without leafhoppers			Total
	No. leaves showing injury					No. leaves showing injury			
	Severe	Slight	None			Severe	Slight	None	
1	6	3	1	10	1a	0	0	10	10
2	8	0	2	10	2a	0	2	8	10
3	3	4	3	10	3a	0	0	10	10
4	5	2	3	10	4a	0	0	10	10
5	5	4	1	10	5a	1	0	9	10

The other five spurs were bagged without insects to serve as controls. They were left undisturbed until August 11, 1924. When the spurs were examined, most of the leafhoppers were dead, but the injury was very characteristic and severe, being much more pronounced than in the open. This was probably due to a prolonged confinement of leafhoppers to the same foliage, which seldom happens in nature, where adult leafhoppers are swiftly moving from one leaf to another and from spur to spur. The control tips

remained healthy, having only one leaf out of fifty that showed the typical symptoms, while two leaves had only a slight trace of yellowing along the veins. This, however, was probably due to a feeding of leafhoppers on these leaves before the tips were caged. Table 1 summarizes the results.

Early in the summer of 1925 the above experiment was repeated, proper precautions being taken to protect the terminal shoots by caging them before the migration of this species of leafhoppers to apple foliage. The spurs were caged on May 20, when apple leaves just opened, and only a very few adult leafhoppers were present on the apple trees. During the second week of June the leafhopper population increased considerably. On June 11, ten protected terminal shoots were taken for the leafhopper feeding experiment. Five of them were colonized with ten adult leafhoppers each, using *Empoasca fabae,* and five shoots were kept protected as controls.

During the course of the experiment, the leaves were examined three times for symptoms. On June 18, most of the leaves showed a considerable downward curling and only a few showed a decided tip burning, with a slight yellowing just preceding the brown burnt areas. On July 3, when the spurs were examined for the second time, they were infested with both the adults and their nymphs of different ages. The spurs increased in length, and practically all of the younger leaves were badly curled downward, showing typical leafhopper feeding injury. Many leaves had their tips and apical margins brown from burning. Practically all of the older leaves and many younger ones displayed the characteristic discoloration along the midrib and lateral veinlets. The symptoms were invariably more progressive on the lower side of the veins than on the upper. Many leaves showed very severe injury. The third and the last examination of the shoots took place on July 14, when it was observed that many leaves were so badly damaged that they were not only discolored along the veins and turned up at the margins, but some of them were entirely dried out and fell off the spurs.

One shoot in this experiment was less affected than others, showing only the feeding injury and very little discoloration along the veins. This fact suggests the thought that probably not all of the leafhoppers of this species are able to produce the characteristic symptoms. It also points to a possible acquisition of virulence by leafhoppers during the feeding on wild hosts before reaching the apple trees. It may be that this tree was resistant to such an attack. This question is not clear, however, and requires further study.

The control shoots had all 51 leaves normal and healthy, without any symptoms of discoloration, curling or burning. Table 2 summarizes the results obtained in feeding of leafhoppers under controlled conditions.

TABLE 2.—*Relation of feeding of Empoasca fabae to apple leaf injury, 1925*

No. of spur	Number of leaves showing a characteristic injury					Total no. of leaves
	Very severe burning	Severe burning and discoloration	Strong discoloration along the veins	Slight discoloration	Only curling due to feeding	
1	4	2	2	1	0	9
2	0	0	0	2	5	7
3	7	3	2	0	0	12
4	5	4	1	0	0	10
5	6	3	1	0	1	11

This experiment, it is believed, definitely indicates that *Empoasca fabae* is responsible not only for curling the leaves due to feeding, but also for the characteristic discoloration along the veins and burning of the foliage, which resembles that of hopperburn of potatoes. Prolonged confinement of leafhoppers on the same foliage considerably intensifies the symptoms.

A few supplementary experiments were carried on in which only one or two leafhoppers were caged over one leaf on each tip, which in turn was also covered with a large bag for protection from other insects. The experiment showed that the downward curling of the young leaves is always associated with the feeding of leafhoppers. Typical discoloration symptoms, although present in the majority of the cases, were not, however, shown in all of them. This indicates the presence of some specific toxic substance or infectious material within the insects, the nature of which is not clearly understood. It was also found that it has a decided downward movement along the midrib and lateral veins, but is restricted to the leaves on which insects were feeding, other leaves of the same tips remaining free from the symptoms.

Similar feeding experiments were conducted with three common apple aphids, colonizing each species separately on leaves of rapidly growing apple tips. The most common aphid in apple orchards in the spring was found to be the apple-grain aphid, *Rhopalosiphum prunifoliae*. Five tips colonized with this species gave negative results so far as the symptoms in *pomi*, which curls the leaves but slightly, hardly tipping the apical ends downward. The rosy apple aphid, *Anuraphis roseus*, was less numerous, but it certainly produced very severe curling and sidewise twisting of the leaves with pronounced pocketing and deformations. Not in a single case, however, were there produced discoloration symptoms in any way comparable to those obtained in feeding of leafhoppers.

ARTIFICIAL PRODUCTION OF SYMPTOMS

Several attempts were made to produce symptoms by artificial inoculations, using inocula prepared by macerating the adults and advanced nymphal stages of *Empoasca fabae* in a small quantity of sterile distilled water in a test tube. The ordinary method of needle inoculation was used, having a small amount of cotton tied to the point of the needle to facilitate the introduction of a sufficient amount of inoculum into each of the needle pricks. Ten fine pricks, aiming to wound the midrib and lateral veinlets, were made on each leaf. Fifty-five leaves, which were protected from insects from May 20 to the end of the experiment, were thus inoculated on June 15, 1925. Three days later on June 18, the inoculated leaves were examined, but no symptoms were noticed with the exception of brown scars in the places of inoculation. On June 23 the second examination was made, and at this time 29 leaves showed progressive discoloration, variable in degree, but similar in all respects to symptoms obtained in the feeding experiments. When the treated tips were examined for the last time on July 3, all but seven of the leaves exhibited the symptoms in varying degrees from slight to severe. Fifty other leaves inoculated with sterile distilled water failed to show any symptoms more than a few dead cells around the needle pricks. This seems to indicate that the adults and advanced nymphal stages of insects contain some active principle which is capable of inducing the symptoms in the leaves.

Two series of inoculation experiments were performed, one between July 10 and August 11, 1924, and another between June 18 and July 14, 1925, by crushing the nymphs and adults on apple leaves, aiding the entrance of insect juice into the plant tissue by needle pricks. Five insects were used to each leaf. Thirty-one per cent of the leaves showed decided symptoms in different degrees. Evidently it is difficult to inoculate the leaves by this method, for the reason that small insects dry too rapidly after they are crushed on the leaves. Sufficient data was obtained, however, to indicate that insects carry some toxic substance or infectious agent to cause the symptoms.

An attempt was made to produce the symptoms by using the sap pressed out of leaves showing different stages of the disease. This work started on July 3 and was in progress until August 13, 1925. The ordinary method of needle inoculation was used, 10 pricks being made in each leaf. Fifty leaves were inoculated with the sap of apparently uninfected leaves as a check. At the close of the experiment all check leaves failed to show injury. Only 3 leaves out of 50 inoculated with the sap of diseased leaves showed decided discoloration. Four leaves showed a faint yellowing, which, however, was not very similar to the typical symptoms. In case of the

potato hopperburn, Eyer (12) obtained positive results in reinoculation made from the diseased tissues, while Fenton and Ressler (13) obtained only negative results, with some browning at the tips of potato leaflets. It is considered that data obtained in this experiment is not sufficiently convincing to warrant any definite conclusions. Yet it is quite possible that a virus from the sap of affected leaves may be transmitted to healthy ones, although giving a relatively very small percentage of infection on account of dilution of infective principle in leaf tissue and the crude artificial inoculation methods employed, as compared with those at the command of a sucking insect.

LEAFHOPPERS AND VIRUS DISEASES OF PLANTS

In connection with these experiments, it may be of interest to note that there are several very important virus diseases of economic plants attributed to feeding of leafhoppers. It will be sufficient to call to mind only a few well known instances in order to appreciate the importance of leafhoppers in transmission and dissemination of virus diseases. The curly-top of sugar beets is produced by punctures of the beet leafhopper, *Eutettix tenella* Baker, as is shown by the works of Ball, Severin, Carsner and others. The potato hopperburn is caused by the potato leafhopper *Empoasca fabae* (Harris) as demonstrated by Ball, Fenton, Eyer, etc. The recent work by Kunkel definitely indicates that the aster leafhopper, *Cicadula sexnotata* (Fallen), is responsible for transmission of the aster yellows. There are a few other cases reported, and no doubt, in the course of time, many more will come to the attention of phytopathologists and entomologists.

In comparing the symptoms and the nature of better known cases, it may be said that there are at least two general types of virus diseases produced by leafhoppers. In case of the curly-top of sugar beets and the aster yellows, the infective principle is diffused and becomes systemic within the plant. The symptoms are usually more apparent in the new growth. In the case of the potato hopperburn, as well as in the symptoms described in this paper, the infective principle is localized in the foliage on which leafhoppers have had opportunity to feed.

It is believed that leafhoppers are equally as, or more important than, the aphids in dissemination of virus diseases, but on account of their agile habits are less suspected as an important factor in the spread of these diseases.

SUMMARY

1. The apple hopperburn symptoms are distinct from the ordinary feeding injury caused by leafhoppers.

2. The typical leafhopper feeding injury is characterized by downward curling of the leaves, which begins at the apex and extends toward the base of the leaves. In this curling the lower surface of the foliage is always rolled in, without a characteristic marginal discoloration along the lateral veins.

3. The apple hopperburn is characterized by marginal yellow discoloration of the leaves which moves progressively downward along the lateral veinlets and midrib. It is invariably more rapid in its development on the proximal side of the lateral veins than on the distal in reference to the petiole of the leaf. In cases of severe injury, the apical margins of the leaves turn brown and often curl upward.

4. Feeding experiments indicate that the potato leafhopper, *Empoasca fabae* (Harris), is not only able to produce the symptoms, but prolonged confinement of insects on apple leaves intensifies the symptoms.

5. These symptoms, observed on apple leaves, strongly resemble the hopperburn of potato, suggesting the existence of some toxic or specific infective principle within the insect.

6. This specific infective principle moves downward in vascular bundles, discoloring the veins, but is localized in the leaves subjected to leafhopper feeding.

7. The symptoms can be produced artificially by needle inoculations with inoculum prepared from macerated adults and advanced nymphal stages of the insect.

8. Crushing the nymphs and adults on apple leaves resulted in a smaller percentage of infection.

9. The sap of affected leaves gives only a very small number of infections, probably due to the crudeness of inoculation technique and to dilution of infective principle in the sap of the plant.

10. The definite association of certain species of leafhoppers with the symptoms; the progressive downward movement of symptoms in the vascular system; and artificial production of symptoms with inocula prepared from the insects and affected leaves indicate that the insect in question is a carrier of some specific infective principle, possibly a virus, in nature.

11. The leafhoppers may be looked upon as a major factor in dissemination and transmission of important virus diseases of plants.

WISCONSIN AGRICULTURAL EXPERIMENT STATION,
MADISON, WISCONSIN

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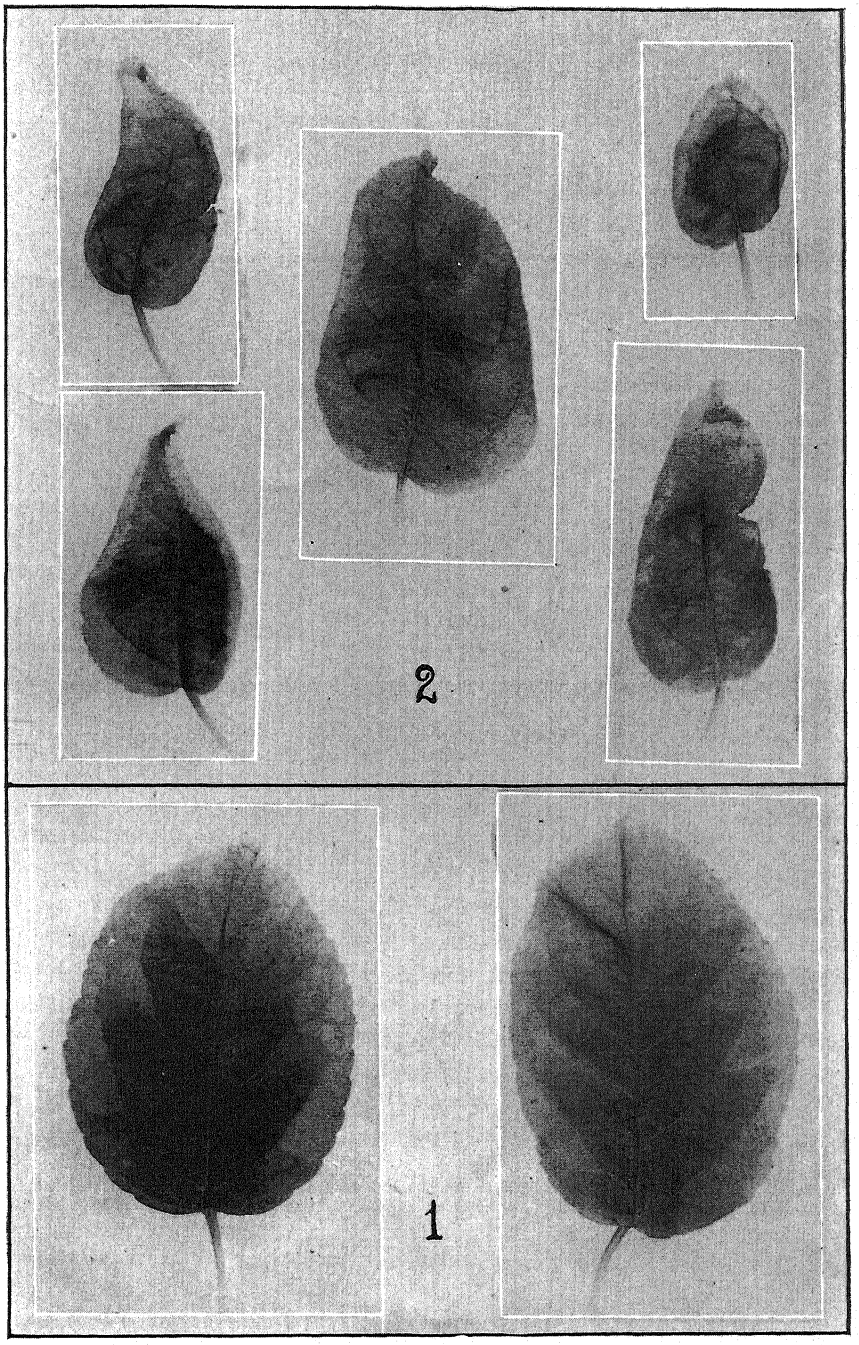
EXPLANATION OF PLATE XXIV

1. Two apple leaves showing a characteristic marginal discoloration, which gradually progresses downward along the midribs and lateral veins in a more or less triangular fashion. It is more rapid on the lower or proximal side of the veins than on the upper or distal side.

2. Several young apple leaves showing advanced stages of "hopperburn," which is characterized by upward curling of leaf margins.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STRAWBERRY "YELLOWS," A DEGENERATION DISEASE OF THE STRAWBERRY[1, 2]

A. G. PLAKIDAS

Strawberry plants in the Central California Coast District have suffered for a long time from an obscure disease. This disease, however, although more destructive in this section than in any other strawberry-growing district, is by no means limited to this region; it occurs in other parts of California, and also in the states of Oregon and Washington.

This trouble has been attributed by the growers to various causes, such as insect injury, too much water, soil conditions, variety degeneration, etc. The evidence at hand shows, however, that this is a new addition to the long list of plant troubles designated as "degeneration," "mosaic," or "virus" diseases; and, as far as the writer is aware, it is the first disease of this nature to be reported on the strawberry. Professor W. T. Horne[3] was the first investigator to recognize the fact that a specific disease was involved and gave a pathological description of it in 1922.

The most conspicuous symptoms of the disease are: (a) a characteristic curling or cupping of the leaves; (b) yellowing of the leaves around the margin and between the larger veins; (c) dwarfing of the leaves, both petioles and blades, and a general stunting of the growth of the entire plant; (d) premature reddening or autumnal color of the older outer leaves. (This is not a very definite symptom, because the leaves of healthy plants also turn reddish brown with age or when affected by frost. However, the leaves of diseased plants turn color earlier than those of healthy plants of the same age.) (e) Appearance of small red dots on the lobes of the margin of very young leaves. (These red dots appear on older leaves, whether healthy or diseased, but they are not present on the very young leaves of healthy vines, while they are always present on the young leaves of diseased ones.) (f) The disease is transmitted through the runners; *i.e.*, every daughter plant will be diseased. (g) The root system appears perfectly healthy during the early stages of the disease.

[1] The term "yellows" has been suggested to the writer by Prof. H. P. Barss, "because of characteristics reminding one somewhat of such virus diseases as aster yellows, peach yellows, etc."

[2] Plakidas, A. G. An obscure new disease of the strawberry in California. Phytopath. 15: 730. 1924. [Abstract].

[3] Rept. of the College of Agr. and Exp. Sta. Univ. of Calif. 122–123. 1921–22.

These symptoms are, of course, characteristic of typical cases. Some of the symptoms may be screened, or not conspicuously manifested under very favorable growing conditions, but again become apparent if the vigorous growth of the plant is checked. Plants may appear to recover at times, but again relapse. Complete recovery of an affected plant never has been noted. The disease is not transmitted through the seed. Seedlings from seed of diseased plants are healthy until they become inoculated.

The following have been considered as possible causes of the disease: the red spider (*Tetranychus telarius* Linn.), adverse chemical conditions of the soil, a soil organism, a virus.

Experiments have shown that no one of the first three is the cause of the disease. The detailed results of these experiments will be presented in a later publication. In this paper only the virus feature of the problem will be considered.

That "yellows" is a virus disease, and that insects act as vectors, is seen from the following circumstantial evidence:

(a) The disease is systemic; all daughter plants coming from diseased mother plants are diseased, and continue to produce diseased plants no matter on what soil they are allowed to grow.

(b) The infection spreads very rapidly and in the direction of the prevailing wind—just what we would expect if insects are the carriers of the infectious principle.

(c) The disease is not transmitted through seed: this is characteristic of diseases of this type.

The results of the following experiments give direct evidence that the disease is caused by a virus. A lot of 40 healthy young plants (variety Banner) in pots on steam sterilized greenhouse soil were divided into five sets of eight pots each and were treated as follows: set A was placed in an isolated spot, away from any other strawberry plants, to be used as check. The plants of set B were placed in another isolated spot and were lightly infested with red spider (*Tetranychus telarius* Linn.) taken from leaves of diseased plants. Similarly, the plants of set C were isolated and were infested each with 10 individual strawberry aphids (*Myzus fragaefolii*) taken from leaves of diseased plants. The pots of set D were placed in a patch of diseased plants and were left unprotected from insects. The diseased plants in the patch were infested with various insects—aphids, white flies, thrips, red spiders, etc. The plants of set E were placed in insect-proof cages in the vicinity of this same patch. This experiment was started the middle of July, 1925.

The results of these experiments are discussed below.

Set A (the control) remained healthy, and are still healthy at the present time. These plants became infested with insects—aphids, white flies, red spider, etc.—but apparently these insects did not come in contact with diseased plants. The plants of sets C and D (*i.e.*, those infested with "infected" aphids, and those left exposed to "infected" insects respectively) began to show "yellows" symptoms about one month after inoculation, and developed all the typical "yellows" symptoms after another month (Fig. 1).

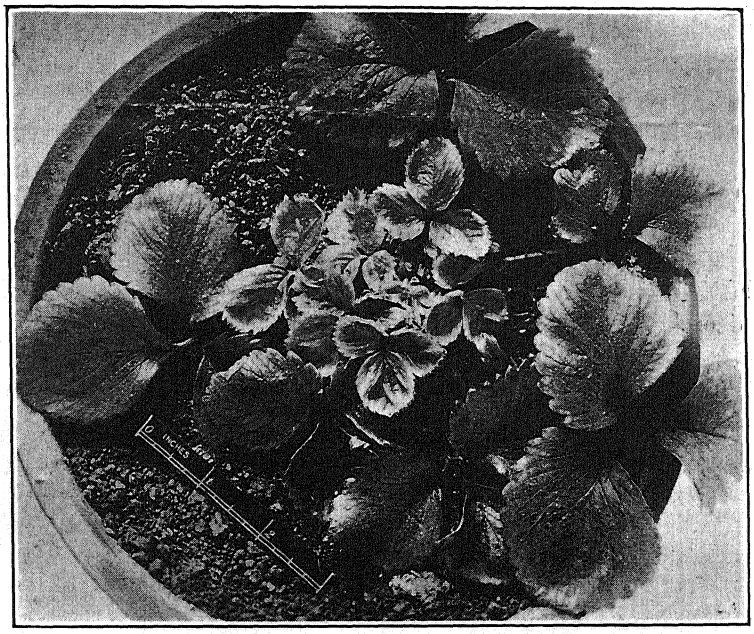

FIG. 1. Young Banner strawberry plant showing typical "yellows" symptoms.

Only 3 out of 8 plants of set B showed symptoms of the disease one month after inoculation; after another five weeks, all plants of this set were diseased. A doubt, however, remains as to whether the red spider inoculated all the plants in this set, or only the three that showed the symptoms first; for soon after the first three plants began to develop the "yellows" symptoms, the plants of this set were infested with strawberry aphids, and it is not improbable that these aphids inoculated the remaining five plants from the first three.

Six of the 8 plants which were placed in cages failed to grow (a severe treatment of nicotine sulphate-oil solution at the time of planting proved too strong for them) but the two that survived developed normally and are still healthy at present.

There is a possibility that this disease is transmissible by direct inoculation with juice of diseased plants, but the evidence at hand at present is inconclusive. Nine out of 11 apparently healthy plants inoculated by means of a hypodermic syringe with extracted juice from leaves of sick plants developed the "yellows," as against 4 out of 11 for each of two control sets—one injected with sterile water, and the other with juice from healthy plants. But, as the plants experimented with were not protected from insects, little, if any, weight can be placed on these results.

Effective insect-proof cages have been constructed, and further experiments are now in progress in order to learn more of the mode and agents of transmission of this disease.

Cytological examination of stained sections of roots, leaves, and petioles of diseased plants so far have failed to show the presence of any intracellular bodies or other abnormalities which are generally found associated with most virus diseases. Further study is being made in this direction, however.

This is a preliminary report of a general study of strawberry diseases carried on in this laboratory under the direction of Professor Ralph E. Smith.

UNIVERSITY OF CALIFORNIA,
DIVISION OF PLANT PATHOLOGY

REVISED METHOD FOR THE QUANTITATIVE DETERMINATION OF SULFUR FUNGICIDES ON FOLIAGE

H. W. Fitch

In a previous article[1] the writer described a method for making a quantitative determination of sulfur fungicides on foliage. The following is a revision of that method and is much more accurate. For each 100 leaves, proceed as follows:

Pour 250 cc. of carbon tetrachloride into a large beaker. Immerse about 35 leaves in the tetrachloride and let stand, with occasional shaking, for 10 minutes. Then remove a few leaves at a time, pulling them out by the stems, and rinse them in a second beaker containing 250 cc. of carbon tetrachloride, carefully shaking off all droplets of solvent before discarding leaves.

Filter the carbon tetrachloride solution through a thin tuft of absorbent cotton in a funnel to remove coarse debris. Collect the filtrate in a 500 cc. flat bottomed flask and distill nearly to dryness on the hot plate through a condensor to recover the solvent. Filter more solution into the same flask, after cooling, until all the solution is filtered and distilled. Rinse the two beakers with Q. S. tetrachloride, passing rinsings through cotton wool, and distill as before.

Add 200 cc. distilled water to the residue in the flask, and then 5 grams sodium peroxide. Heat on the hot plate for 2 hours. Cool and add an additional 5 grams of peroxide. Heat an additional 2 hours. Cool and make decidedly acid to methyl red by addition of concentrated hydrochloric acid C.P. Heat about 1 hour on the hot plate and cool. Then place in the flask a small quantity of dry asbestos fiber, such as is used for Gooch crucibles, and shake well. The asbestos fiber will collect all flocculent waxes, etc., which are thrown out on acidifying.

Next prepare a Büchner funnel as follows:

Place in funnel two filter papers, wet, and sucked dry. Then form a fairly thick mat of asbestos fiber on the filter paper and suck dry. Next place a small watch glass on the asbestos mat, raising one edge by placing a short length of glass rod under it.

[1] Fitch, H. W. Quantitative determination of sulphur fungicides on foliage. Phytopath. 15: 351–354. 1925.

The acid solution may now be filtered readily, pouring it on the watch glass in order not to disturb the asbestos mat. Wash well with three 75-cc. portions of distilled water.

Now neutralize the caustic soda until the solution is only faintly acid to methyl red, and determine barium sulfate in the usual way. When filtering the barium sulfate precipitate, test wash water with silver nitrate until free from chlorides.

Note: All re-agents, especially the sodium peroxide, must be free from sulfates.

For larger quantities of leaves, the same procedure is employed, increasing the quantities of re-agents used accordingly.

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PHYTOPATHOLOGICAL NOTES

The Sylloge Fungorum vol. 23.—The twenty-third volume of Saccardo's invaluable Sylloge Fungorum, issued by Dr. Alex. Trotter, treats of the Basidiomycetes and contains also, besides two pages of necrology and the announcement that volumes 24 and 25 are forthcoming, a brief tribute to Saccardo and a bibliography of his writings numbering 238 titles.

To the Hymnomycetes are given 583 pages; to the Gastromycetes 23 pages, to the Ustilaginaceae 27 pages, and to the Uredinales 317 pages.

Some conception of the records given may be gleaned from the fact that 82 numbers are given to *Uromyces,* 331 to *Puccinia,* 36 to *Ravenelia,* 43 to *Ustilago,* 108 to *Polyporus,* and 66 to *Clitocybe.* The mode of treatment is that of earlier volumes of the Sylloge.—F. L. STEVENS.

Glomerella cingulata.—In a recent article (Mix, A. J., Anthracnose of European privet. Phytopath. **15**: 261–272. 1925), the name of the fungus causing privet anthracnose was given as *Glomerella cingulata* (Atk.) Spaulding and von Schrenk. This should have been: *Glomerella cingulata* (Stoneman) Spaulding and von Schrenk.—A. J. MIX.

PHYTOPATHOLOGY

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A COMPARATIVE STUDY OF THE MOSAIC DISEASES OF CUCUMBER, TOMATO, AND PHYSALIS

M. N. WALKER[1]

INTRODUCTION

The results of cross-inoculation experiments with the mosaic diseases occurring on various plants have shown that many of these diseases affect a wide range of hosts. The work of Doolittle (11, 12) and of Elmer (15) has shown that the mosaic occurring on a given host may be transmitted to plants of widely separated families and would seem to indicate that a number of mosaic diseases may have the same causal agency. At the same time, Jagger (17), working on the mosaic disease of cucumber, has described what he believes to be two distinct mosaic diseases of this host. Allard (3) states that there are at least two distinct mosaic diseases in the genus *Nicotiana*, and Schultz and Folsom (19) have described and named seven different virus diseases in the genus *Solanum*. We have, therefore, two lines of evidence, one indicating an extended host range for individual mosaic diseases, and the other indicating the existence of a number of distinct types of mosaics on a single host species. From the latter possibility the question arises as to whether, for instance, we may consider the mosaic disease on cucumbers infected from mosaic milkweeds as cucumber mosaic, or as milkweed mosaic on the cucumber. If we follow the latter system, the whole field of mosaic investigations becomes tremendously complicated, since it is obviously impossible to determine the original source of a single mosaic disease.

Where the symptoms resulting from successful cross-inoculations are identical, it appears easier to accept the theory of a single mosaic disease which is capable of infecting a large number of hosts than that of a number of distinct mosaic diseases each with the same extended host range. From the accumulated evidence of successful cross-inoculations, the number of distinct mosaic diseases would seem to be less than is sometimes stated or

[1] Presented in partial fulfillment of the requirements for the degree of Ph.D. at the University of Wisconsin. The writer is indebted to Dr. L. R. Jones, of the Department of Plant Pathology, of the University of Wisconsin, and to Dr. S. P. Doolittle, of the Bureau of Plant Industry, at whose suggestion the problem was undertaken, for advice and suggestion during the course of the work.

implied, but in some cases this evidence alone seems hardly sufficient to fully establish a single causal agency for the mosaics occurring on the various hosts.

A comparison of the results of experiments by Allard and other workers on the properties of the virus of tobacco mosaic with those obtained by Doolittle in work on the virus of cucumber mosaic shows a decided difference in the reactions of these viruses to certain vital tests. These tests seem conclusive in showing two distinct causal principles for the two diseases. Doolittle (12), however, has shown that, by using the pepper as an intermediate or bridging host, it is possible to make successful cross-inoculations from tobacco to cucumber and vice versa; Elmer (15) reports direct infection of tobacco with cucumber mosaic. These facts suggested that a comparative study of these apparently differing viruses, in connection with cross-inoculation studies, might prove of value in interpreting the results of the cross-inoculations mentioned. Two possibilities arose: first, that, were the differences in the viruses of the two diseases actual, the virus in the juices of plants of one species infected from plants of another species would show the properties of the virus of the infecting host. That is, should cucumber plants be inoculated from mosaic tobacco plants, the properties of the extract from these mosaic cucumber plants would be the same as those of the extract from mosaic tobacco plants, and would indicate that the diseases of the two hosts were distinct and caused by different agencies. Secondly, that, were the differences only apparent and dependent on the environments within the different hosts, such changes would occur in the virus of one host, when transferred to another, as to make it appear identical with the virus of the second host. In such a case, the extract from cucumber plants inoculated from mosaic tobacco would react in the same manner to different tests as the extract from cucumber plants infected from other mosaic cucumbers. In either case it appeared that the results of such tests would give more definite evidence as to the identity or dissimilarity of the two diseases. Representative mosaic hosts from the Cucurbitaceae and the Solanaceae, together with a suitable intermediate host, were selected for this work. Since the mosaic disease of tomato is apparently identical with tobacco mosaic, and because the properties of the virus of tomato have been little studied, this plant was used as the representative of the Solanaceae. In recent work by the writer (20), the cultivated ground cherry (*Physalis pubescens*) has been shown to be an intermediate host in cross-inoculations from the tomato to the cucumber; it was, therefore, chosen as the intermediate host. The cucumber was used as the third host plant. It was planned to determine first the reactions of the infective principle of the mosaic disease of each of these hosts to certain tests, and then to determine the changes that might occur in the virus from one host inoculated with the

virus of another plant. The difficulty lay in deciding on what might be taken as the typical virus of any one plant, since, on account of the general transmissibility of many of the mosaic diseases, it is impossible to know positively the original source of infection of a single mosaic disease. The disease on tomato, for instance, might have originally come from tobacco, or any other solanaceous host; consequently the infective principle in the extracted juices of a mosaic plant cannot be called the virus of the disease of that plant in the sense that it is specific for that particular host. Specific in this sense is more or less inaccurate, for apparently all mosaic diseases may be inoculated to hosts other than that on which the disease occurs, but the term is used here to denote a virus that might be considered typical or usual on a single species of host. As a means of obviating this difficulty, it was assumed that plants naturally infected with mosaic would be as nearly typical as any that might be obtained; and, following this assumption, stocks of mosaic plants of the species used in the experiments were obtained by inoculations from naturally infected tomatoes, ground cherries, and cucumbers; and all the extracts used in the work came from these stocks. It seemed best for the sake of accuracy to avoid as far as possible the use of the term virus, which has often been used for the expressed juices of mosaic plants, and to substitute the term extract, which can be qualified by stating the immediate source of infection on the plants from which the extract is taken. The use of some such general term was especially necessary in the case of extracts from plants of the same species inoculated from two or more different sources. For example, it is obvious that the extracts from tomato plants infected from tomatoes and from ground cherries could not both be called the virus of tomato mosaic; the latter would have to be called the virus of ground cherry mosaic extracted from tomatoes. The term virus, therefore, is only used in the general sense of the infective principle or causal agent, and in quotations from other workers.

For the purposes of comparison, tests were made with the extracts from different host plants to determine: (a) the longevity of the infective agent; (b) its resistance to drying; (c) its resistance to heat; (d) its resistance to the higher percentages of alcohol; (e) its resistance to dilution; and (f) its ability to pass through the finer-pored filters. Various things interfered to prevent the completion of the work in the desired detail, and after some preliminary experiments the last three points were found to give results so nearly identical in the case of the juices of all three hosts that they were of little value as bases of comparison; consequently they were not followed as closely throughout the work as in the beginning.

Since the tests selected were primarily to furnish comparisons rather than to determine the exact properties of the virus in the different extracts, the absolute properties have in no case been accurately determined. When

one extract, for instance, showed a decided difference from another, that was considered sufficient for the present purposes, and no attempt was made to follow the tests to their exact endpoints. In the light of results set forth in the succeeding pages, it is a question whether such properties are of great value in determining the fundamental nature of the infective entity of the mosaic diseases, for it is shown that it is possible to change radically the properties of a particular virus by transferring it to another host. Thus, such tests represent only the reaction of the virus when combined with the extracted juices of a particular host and are not necessarily indicative of the fundamental nature of the infective principle.

REVIEW OF STUDIES ON THE PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACT FROM MOSAIC TOBACCO PLANTS

The work that has been done on the properties of the infective principle in the extracted juices of mosaic tobacco plants represents the greater part of the virus studies that have been made with the mosaic diseases. They have served, therefore, as a source of suggestion in outlining comparative tests for the diseases studied here. For this reason it seemed best to review the work on the virus of tobacco mosaic and to make incidental comparison of this virus with those specifically studied here. The literature cited includes only those papers that have direct bearing on this phase of mosaic disease investigations. The most thorough and extensive work with the tobacco virus has been done by Allard, and his work is the basis of the major portion of the summary of the properties of the tobacco virus. The work of other men is cited under individual headings of the summary when their results have helped to determine the particular property there mentioned.

Resistance to ageing. The virus of the mosaic disease of tobacco has been found by all workers (1, 4, 5, 8, 9) to remain infective for a considerable period of time after extraction from the plant. Both with and without preservatives the extract from mosaic tobacco plants has been shown to remain infective for as long as three years.

Resistance to drying. Several workers (1, 4, 5, 8, 9) have noted the resistance of the virus of tobacco mosaic to prolonged periods of drying at air temperatures. Both air-dried leaves and the residue from evaporation of the extracted juice have proved infective when taken up in water and inoculated into young plants two years later.

Thermal death point. A considerable range of temperatures has been suggested by different writers (1, 4, 5, 8, 9, 14) in naming a thermal death point for the virus of tobacco mosaic. No two men have used the same time for treating the virus at a given temperature, and this fact makes the selec-

tion of a thermal death point difficult. The range of temperature is from 80° to 100° C., and it has been found that the tobacco virus from different sources varies somewhat in resistance to heat. The most probable thermal death point seems to lie between 85° and 90° C. for a 10 minute period, corresponding to the time used for heat treatment in the present work.

Resistance to treatment with alcohol. In the determination of the resistance of the tobacco virus to different percentages of alcohol, the time element makes definite statements difficult. Allard (4) has shown the virus to be capable of withstanding percentages of alcohol up to 50 per cent for several days; higher percentages rendered the virus inert in varying lengths of time. In any case, the virus shows a striking resistance to percentages of alcohol which are fatal to bacteria in the vegetative state; and, even with the higher percentages, some time is necessary to effect an attenuation of the virus.

The effect of dilution. Allard (2) has shown that dilution of the virus of tobacco mosaic at the rate of one part to one thousand parts of water does not attenuate the virus; although higher dilutions reduce its infective power.

Ability of the infective principle to pass filters. It has been shown by several different workers (1, 4, 5, 8, 9, 10, 13) that the virus of tobacco mosaic is capable of passing through Berkfeld and Chamberland filters without losing its infective powers. Certain filters have been found capable of retaining the infective particles (parchment shells, layers of agar and powdered talc), and, by using a graded series of filters, Duggar (13) has estimated the size of the infective particles of the mosaic disease of tobacco to be in the neighborhood of 30 millimicrons.

STUDIES ON THE PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACT FROM MOSAIC TOMATO PLANTS

Little work has been done on the properties of the virus of tomato mosaic, only one paper mentioning such work. Westerdijk (22) reports that the thermal death point for the virus lies between 50° and 100° C., and that it is quite resistant to drying. Since so little work has been done with the virus of tomato mosaic, it was necessary to make a study of the virus before comparing it with viruses from tomato plants inoculated from different sources. The study was limited to the six points selected as bases for comparison. The extracts for all these experiments were taken from mosaic plants infected from the same source, a stock of mosaic plants developed from tomato plants found naturally infected in the greenhouse, and were obtained by first grinding the plants in a food-chopper and then squeezing the juice from the pulp so obtained through a piece of cloth. The variety of tomato used in all the present experiments was Bonny Best. When used

for inoculations the plants were stocky, deep green in color, and averaged about six inches in height.

Resistance to ageing. It was found that the extract from mosaic tomato plants does not lose its infective power after a considerable period of time. In the experiments, extracts were kept for varying lengths of time in small, stoppered flasks, with no effort made to prevent fermentation. They were then used to inoculate healthy young tomato plants. Inoculations were made by inserting the inoculum in two stem incisions, the lower of which was usually in the wound made by removing a leaf, and by pricking the inoculum into the younger leaves in many places. Inoculations with the extract kept 138 days produced infection in a single case. The results of this experiment, and others, are shown in table 1.

TABLE 1.—*The resistance to ageing of the infective principle in the extract from mosaic tomato plants*

Date inoculated 1924	Age of virus in days	No. plants inoculated	No. plants diseased	Date observed, 1924
April 17	38	5	2	April 28
April 21	4	8	7	May 12
April 23	6	8	6	May 12
April 17	38	5	2	April 28
July 25	138	6	1	August 3

Resistance to drying. The work of Westerdijk (22) on the resistance of the tomato virus to drying was sufficient to show a difference from the virus of cucumber. Although the work was not extensive, it showed that the virus was somewhat resistant to drying, which, were it even in the slightest degree, would render it unlike the cucumber virus, which has been shown by Doolittle (11) to be unable to withstand drying.

The work of the writer also shows that the infective principle in the extract from mosaic tomato plants is capable of withstanding drying, although the limit of this endurance was not determined. It is probable, however, that its resistance is the same as that of tobacco, but, as one experiment showed a resistance of sufficient duration to distinguish it in this point from the extract from mosaic cucumber plants, this detail was omitted. In this experiment, 3 out of 6, and 2 out of 5 plants became infected when inoculated with material dried for 4 and 30 days, respectively. In a series of controls in which the fresh juice served as inoculum, 4 out of 5 plants became infected.

Resistance to heating. In experiments on the resistance of the viruses of the various mosaic diseases to heating, the same method is used as that described by Kendrick (18) for thermal death point work. Test tubes containing 10 cc. of the juices expressed from mosaic plants were suspended in water brought to the desired temperature and allowed to remain for 10 minutes. They were then removed and immediately suspended in cold water to cool. The juices then were used to inoculate young plants, using the juice heated to the highest temperature first, and so on down the entire series to the control inoculations made with the unheated juice.

Because of the wide range of temperatures indicated in Westerdijk's paper (22), a more thorough study of this question was undertaken. The results in table 2 show that the thermal death point of the tomato virus lies within a narrower range than that suggested by Westerdijk. The virus became innocuous in all experiments when heated to temperatures between 85° and 90° C., with the exception of one series in which one plant became infected after treatment of the virus at 95° C.

The inability to find a definite thermal point for the infective principle in the extracts from mosaic plants means little when it is considered that one is not working with the virus or infective principle alone, but with the variable medium of the plant juices as well. The possibility of there being decided differences in the infective power of the virus is shown in previous experiments by the writer (20, 21), who has found an apparent decrease in the virulence of the extract from mosaic plants at different times of the year. It is impossible to say what part of this may be attributed to changes in the actual infective principle, and what part to changes in the composition of the plant juices at different times, but in any case this work indicates that it is difficult to determine the definite thermal relationship or any other property of something hidden in a fluid extract, the composition of which varies with the source and the season. No definite thermal death point can be ascertained until the infective entity is isolated from the many other substances in the extracts from mosaic plants which apparently exert such a marked influence on the behavior of the virus.

Resistance to treatment with alcohol. In testing the effect of treatment with alcohol on the extracts from mosaic plants, small quantities of the extracted juices were brought to various percentages of alcohol by the addition of absolute alcohol, and allowed to stand for one hour. In all treatments with alcohol a light flocculent precipitate occurred which was separated from the supernatant liquid by centrifuging. This precipitate then was taken up in a small quantity of water and used to inoculate young, healthy plants. No inoculations were made with the green supernatant alcoholic solution.

TABLE 2.—*The effect of temperature on the infective principle in the extract from mosaic tomato plants*

Date inoculated, 1924	Temperature in degrees C. (constant for 10 min.)	No. plants inoculated	No. plants diseased	Date observed, 1924
Feb. 14	65	4	0	March 3
do	70	4	2	do
do	75	4	0	do
do	80	4	0	do
do	85	4	2	do
do	90	4	0	do
do	Unheated extract (Control)	4	2	do
March 3	65	5	3	March 19
do	70	5	4	do
do	75	5	3	do
do	80	5	2	do
do	85	5	1	do
do	90	5	0	do
do	95	5	0	do
do	100	5	0	do
do	Unheated extract (Control)	5	1	do
March 10	65	4	4	March 19
do	70	4	4	do
do	75	4	3	do
do	80	4	4	do
do	85	4	2	do
do	90	4	0	do
do	95	4	0	do
do	Unheated extract (Control)	8	7	do
March 21	65	4	4	April 8
do	70	4	3	do
do	75	4	2	do
do	80	4	2	do
do	85	4	0	do
do	90	4	0	do
do	95	4	0	do
do	100	4	0	do
do	Unheated extract (Control)	8	6	do
March 27	75	6	2	April 14
do	80	6	5	do
do	85	6	4	do
do	90	6	1	do
do	95	6	1	do
do	Unheated extract (Control)	12	10	do

TABLE 3.—*The effect of alcohol on the infective principle in the extract from mosaic tomato plants*

Date inoculated, 1924	Per cent alcohol used	Time	No. plants inoculated	No. plants diseased	Date observed, 1924
April 17	33	1 hr.	5	3	April 28
do	75	do	5	4	do
do	80	do	5	1	do
do	85	do	5	2	do
do	95	do	5	1	do
April 25	80	do	8	8	May 10
do	85	do	8	7	do
do	90	do	9	8	do
do	95	do	8	8	do
do	Untreated extract (Control)	do	8	8	do

In the experiments of this nature with the expressed juices from mosaic tomato plants, it was shown that there is a marked resistance to alcohol of the infective principle in these juices. Infection followed inoculation with the precipitate from treatment with all percentages of alcohol used up to the highest, 95 per cent. The results of inoculations with the precipitate from different percentages of alcohol are given in table 3.

The effect of dilution. A few experiments were made with the extract from mosaic tomato plants to determine to what extent it might be diluted

TABLE 4.—*The effect of dilution on the infective principle in the extract from mosaic tomato plants*

Date inoculated, 1924	Dilution	No. plants inoculated	No. plants diseased	Date observed, 1924
April 17	1:20	5	5	April 28
do	1:200	5	4	do
do	1:1,000	5	0	do
do	1:2,000	5	1	do
do	1:20,000	5	0	do
do	Undiluted extract (Control)	5	2	do
April 28	1:100	8	7	May 10
do	1:1,000	8	4	do
do	1:10,000	8	2	do
do	Undiluted extract (Control)	8	8	do

before becoming attenuate. It was found that the infective power of the virus was not reduced greatly until after a thousand parts of water had been added.

Dilution experiments furnished no opportunity for comparing the viruses studied in the present work, for all experiments of this sort gave approximately the same results. Of the six points used as bases of comparison, this one is probably the most indefinite and is subject to greater variation than any of the other points. The results of dilution experiments with the extract from mosaic tomatoes appear in table 4.

Ability of the infective principle to pass filters. It has been impossible to contrast the infective principles from various mosaic diseases by the results from filtration experiments, because the results of all tests were so nearly alike. The greater portion of the present experiments have been made with Berkfeld filters of three grades: coarse, medium, and fine, which are lettered V, N, and W respectively, and which were of the 1″ x 6″ size. A few experiments also have been made with Chamberland filters, grades F, coarse, and B, fine, but results have been no more conclusive than those obtained with Berkfeld filters. The fact that the filtrates from Berkfeld filters were all infective, and the fact that the filtrates from Chamberland filters gave such low percentages of infection, precluded the use of these filters for routine comparisons of the viruses.

The results of filtrations with the extract from mosaic tomato plants show that the infective principle in it is capable of passing through all grades of Berkfeld filters with little loss of infective power. These results are set forth in table 5.

TABLE 5.—*The results of inoculating healthy tomato plants with the filtered extract from mosaic tomato plants*

Date inoculated 1924	Filter used	No plants inoculated	No. plants diseased	Date observed, 1924
March 10	Berkfeld W	8	4	March 24
April 14	do W	16	8	April 26
do	do N	8	7	do
do	do V	8	6	do
do	Unfiltered extract (Control)	8	8	do
April 16	Berkfeld W	8	7	April 28
do	do N	9	9	do
do	do V	8	7	do
do	Unfiltered extract (Control)	8	8	do

STUDIES ON THE PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACT FROM MOSAIC GROUND CHERRY PLANTS

The preliminary tests with the extracts from mosaic ground cherry plants showed a marked similarity to the tests on tomato extract. In some cases the results were not so extensive as those with the tomato, but the results indicate that the infective principles of these diseases behave in the same manner.

Resistance to ageing. The infective properties of the extract from mosaic ground cherry plants is apparently little impaired by being held for long periods of time without preservatives. The ultimate range of time necessary to effect attenuation in the virus has not been determined, but it was found infectious by means of inoculations to healthy young ground cherry plants after 69 days. Three tests were made in each of which 6 plants were inoculated with extracts 13, 28, and 69 days old. In each of the first two experiments, 4 plants became infected; and in the third, 2.

Resistance to drying. In a limited number of experiments it was shown that the infective principle in the extract from mosaic ground cherry plants resists drying to a degree comparable with that of the infective principle in the extract from mosaic tomato plants. Two series of experiments have shown that the dried extracts remain infectious for at least 23 days.

Resistance to heating. In experiments in which the extract from mosaic ground cherries was heated to different temperatures and then used to inoculate young plants, it was shown that the infective principle, or virus, in the extract is capable of withstanding heating to the same degree as the viruses of tomato and of tobacco mosaic. The virus remained infective after treatment for ten minutes at 85° C. but became innocuous a little above this temperature. Table 6 presents the results of these experiments.

TABLE 6.—*The effect of different temperatures on the infective principle in the extract from mosaic ground cherry plants*

Date inoculated	Temperature in degrees C. (constant for 10 min.)	No. plants inoculated	No. plants diseased	Date observed
May 22, 1924	65	5	5	June 1, 1924
do	70	5	5	do
do	75	5	5	do
do	80	5	4	do
do	85	5	2	do
do	90	5	0	do
do	95	5	0	do
do	Unheated extract (Control)	5	5	do

Resistance to treatment with alcohol. Experiments with the extract from mosaic ground cherries showed that the infective principle in the extract behaves in the same manner toward high percentages of alcohol as those from mosaic tomato and mosaic tobacco plants. When the virus was treated with 95 per cent alcohol for one hour, infection occurred in 3 out of 8 inoculated plants and similar results were secured with 50, 60, 70, and 80 per cent alcohols.

The effect of dilution. Tests on the relation of the infective principle from mosaic ground cherry plants to dilution show that it remains infective in as high dilutions as the viruses of tobacco and tomato mosaic. It was found in a single series of inoculations with the dilutions 1:10, 1:100, 1:1,000, and 1:10,000 that 4 out of 5, 2 out of 5, 3 out of 5, and 1 out of 5 plants, respectively, became infected. In the control inoculations with the undiluted extract, 3 out of 5 plants became infected.

Ability of the infective principle to pass filters. The infective principle in the extract from mosaic ground cherry plants appears to lose little of its infective nature after passing through the various grades of Berkfeld filters. Inoculations with the filtrate from all grades of Berkfeld filters, from coarse to fine, resulted in about the same percentage of infection; this is shown in table 7.

TABLE 7.—*Results of inoculating healthy ground cherry plants with the filtered extract from mosaic ground cherry plants*

Date inoculated, 1924	Filter used	No. plants inoculated	No. plants diseased	Date observed, 1924
April 26	Berkfeld W	8	5	May 12
do	do N	8	5	do
do	do V	8	6	do
May 28	do W	5	2	June 14
do	do N	5	2	do
do	do V	5	2	do
do	Unfiltered extract (Control)	5	4	do

PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACT FROM MOSAIC CUCUMBER PLANTS

The rapid loss of the pathogenicity of the extract from mosaic cucumber plants after the juices are expressed has forced the writer to fall back, to a large extent, on other work for positive data concerning the properties of the infective principle in the extract. All the experiments conducted by the writer with the extract from mosaic cucumber have given unsatis-

factory results. The small amount of infection in some experiments, and the total lack of infection in others, has allowed only conclusions concerning the short-lived nature of the virus, which did not permit the handling necessary for the different tests.

Doolittle (11), to whose paper the writer is indebted for positive data on the properties of the infective principle in the extract from mosaic cucumber, reports a similar difficulty in much of his work with this extract.

Resistance to ageing. Doolittle reports that: "Repeated tests have shown the juices of mosaic plants of cucumber, squash, pumpkin, muskmelon, and *Micrampelis lobata* are never infectious for more than three to five days and in most cases lose their virulence within 24 to 48 hours." Preservatives and low temperatures were found to prolong in no way the period of virulence of the infective principle from these plants.

The results of the writer's experiments, tabulated in table 8, are in accord with those of Doolittle's experiments, and show the inability of the infective principle in the extract from mosaic cucumbers to withstand ageing.

TABLE 8.—*The resistance to ageing of the infective principle in the extract from mosaic cucumbers*

Date inoculated, 1924	Age of virus	No. plants inoculated	No. plants diseased	Date observed, 1924
July 6	1 hour (Control)	8	2	July 16
July 19	do	6	2	Aug. 1
July 7	24 hours	4	1	July 16
Aug. 2	do	6	0	Aug. 11
July 8	48 hours	4	0	July 16
March 10	6 days	8	0	March 19

Resistance to drying. In the case of experiments on the resistance of the virus to drying, Doolittle states that: "As the juices of mosaic plants rapidly lose their power of infection with age, it would be expected that drying would also remove this infective character. This has been the case with the tissues of mosaic cucumber plants." In no case did Doolittle find infection to follow inoculation either after drying down the virus at room temperatures and then taking it up in water, or after drying down leaves and later pulverizing them in water. Results of a single experiment by the writer also showed that the extract from mosaic cucumber plants will not withstand drying for 24 hours. From the results in connection with Doolittle's work, and the results set forth later, on the effect of drying on the extract from cucumber plants infected from different sources, it is

evident that the infective principle in the extract from mosaic cucumbers is incapable of withstanding drying. Six plants inoculated with the dried extract from mosaic cucumbers remained healthy, while two out of six other plants inoculated with the fresh extract became infected.

Resistance to heating. According to Doolittle (11), the expressed juices of mosaic cucumber plants lose their power of infection when heated above 70° C. The writer's experiments on the relation of temperature to the infective power of the virus of cucumber mosaic were almost entirely negative. In three experiments carried on in March, July, and August, 1924, to determine the effect of temperature on the infective principle in the extract from mosaic cucumber plants, the extract was exposed for 10 minutes to temperatures from 50° to 80° C. in 5° intervals. The treated extract was then inoculated into series of 4 to 6 plants, 74 plants in all being inoculated. All tests with the heated extract were negative except one, in which 1 out of 6 plants inoculated with the extract heated to 75° C. became mosaic, but a single infection is of little value as data. In one trial the check gave 1 mosaic plant, in another 2, and in a third none. It will be noted in the data given that a very small percentage of infection followed inoculation with the unheated juice; the controls and the negative data merely furnished an idea as to the rapidity of degeneration of the infective principle in cucumber mosaic.

Resistance to treatment with alcohol. In an endeavor to isolate a possible causal enzyme from juice extracted from mosaic cucumber plants, Doolittle treated the extracted juices with different percentages of alcohol. The time of treatment is not mentioned, but in no case was infection obtained when the alcohol precipitates were taken up with water and used to inoculate young cucumber plants. Of this work Doolittle says, "A few attempts were made to isolate a possible causal enzyme from the juices of mosaic cucumber plants. The fact that the juices of such plants usually lose their power of infection within 24 to 48 hours has proved an almost insurmountable obstacle in such work."

The writer, in a single series of experiments, obtained no infection following treatment of the extract from mosaic cucumber plants with different strengths of alcohol, but inasmuch as no infection appeared in the controls it is probable that the killing occurred because of the time taken for the treatment, and the results may again be interpreted only to show the short-lived nature of the virus.

The effect of dilution. Doolittle (11), in speaking of dilution experiments with cucumber virus, says, "These experiments show that dilutions of 1:1,000 are as potent as undiluted solutions, but while infections may result from those of 1:10,000 they have never taken place at higher dilutions."

Inoculations made with dilutions of the extract from mosaic cucumber plants gave no infection in the experiments of the writer. The control inoculations in all experiments were made following the other inoculations in order to eliminate the possibility of confusion in the results arising from the difference in time of extraction of the juices and the inoculations, for should infection result in the controls it could be surmised that the time of treatment for the remaining part of the experiment was not the factor responsible for any diminution in percentage of infected plants. In these experiments controls were made before and after the dilutions were made, but no infection was obtained in any of the plants. The absence of infection, even in the controls made a half hour after extraction, cannot be definitely explained, for there can be no doubt as to the plants from which the extract was taken being affected with mosaic.

Ability of the infective principle to pass filters. Doolittle found little loss in the infective nature of the extract from mosaic cucumber plants after passage through Berkfeld filters. He found, also, that all types of Chamberland filters are capable of retaining the infective entity of cucumber mosaic. In only 4 cases out of 60 inoculations was the filtrate from Berkfeld filters found infective to young cucumber plants. Since the infection occurred following inoculation with the filtrate from the finest grade of Berkfeld, W., it is safe to infer that the virus is filtrable through the coarser grades of Berkfeld, N. and V. A few inoculations with filtrates from Chamberland filters gave no infection. Since a low percentage of infections resulted in these experiments in the control inoculations made with extract kept for approximately the same length of time required for filtration, it is probable that the time factor was more important in destroying the infectiousness of the extracts than the filtration.

PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACTS FROM CROSS-INOCULATED PLANTS

The infective principles in the extracts from mosaic tobacco, tomato, and ground cherry plants have been shown in the preceding pages to react in an almost identical manner in their ability to successfully withstand certain vital tests. It is also shown that the infective entity in the extract from mosaic cucumber plants is incapable of withstanding the same tests in a comparable degree.

On account of the similarities of the reactions of the tomato and ground cherry extracts it was not thought possible to discern changes in the properties of the extract from one of these plants when inoculated with the extract of the other. For instance, in the case of the thermal death point, which was shown for both the extract from mosaic tomatoes and from mosaic ground cherries to be in the neighborhood of 85° C., it will be seen

that the possibility of a discernible change was less than in the same test on the extracts from mosaic ground cherries and from mosaic cucumbers, where there is a difference of 15° C. Consequently the chief comparisons were made with the extracts obtained from cross-inoculations between cucumber and ground cherry. The extracts of these two hosts appeared very unlike in resistance to tests on ageing and drying, and the cross-inoculations between cucumber and ground cherry more readily were made than those between cucumber and tomato, since the latter crosses required an intermediate host. After some preliminary work it was found that certain of the tests listed in the introduction did not offer such good opportunities for comparing viruses from different plants; consequently they were either slighted or dropped. The tests on ageing, drying, and heating offered the best possibilities of contrast, and, since the work was outlined with the idea of obtaining comparisons rather than determining the exact properties of individual viruses, these points have been emphasized.

PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACT FROM TOMATO PLANTS INOCULATED WITH THE EXTRACT FROM MOSAIC GROUND CHERRY PLANTS

Resistance to ageing. Experiments were made with the extract from tomato plants infected from mosaic ground cherry plants to determine the resistance of the virus therein to ageing. It was found that infection occurred following inoculation of young tomato plants with the virus 40 days after extraction. This period is not as long as those used in the tests on the infective principles taken as typical for tomato and ground cherry mosaic, but indicates that the viruses, or infective principles, in these two hosts behave in the same manner. In tests with extracts 1, 2, 8, 14, and 40 days old, in which 6 plants were inoculated with each extract, 6, 6, 3, 1, and 2 cases of infection resulted, respectively.

Resistance to drying. The results of a single series of inoculations show that the resistance to drying of the infective principle in the extract from mosaic tomatoes infected with the extract from mosaic ground cherry plants is similar to that of the infective principle in the extract from mosaic tomatoes infected with the extract from tomatoes. In this experiment 4 lots of 6 plants each were inoculated with extracts dried for 1, 2, 8, and 14 days; 6, 6, 3, and 2 infections resulted, respectively.

Resistance to heating. Effects of different temperatures on the extract from tomato plants inoculated with the extract from mosaic ground cherry plants are apparently identical with those on the extract from mosaic tomato plants or from mosaic ground cherry plants inoculated with the extract from plants of their own kind as the results in table 9 show. In all

cases the infective principle of the mosaic disease of such cross-inoculated plants is rendered inert between 85° and 95° C.

TABLE 9.—*The effect of temperature on the infective principle in the extract from mosaic tomato plants inoculated with the extract from mosaic ground cherry plants*

Date inoculated, 1924	Temperature in degrees C. (constant for 10 min.)	No. plants inoculated	No. plants diseased	Date observed, 1924
July 18	70	6	5	July 29
do	75	6	5	do
do	80	6	6	do
do	85	7	5	do
do	90	6	2	do
do	95	6	0	do
do	Unheated extract (Control)	6	6	do

Resistance to treatment with alcohol. The higher percentages of alcohol have no more effect on the infective principle in the extract from tomato plants infected with the extract from mosaic ground cherry plants than on the infective principle in the extract from tomatoes infected from other tomato plants, or that contained in the extract from ground cherry plants inoculated from mosaic ground cherries. In this experiment 4 lots of 6 plants each were inoculated with extracts treated for one hour with 70, 80, 90, and 95 per cent alcohol; 5, 5, 5, and 4 plants, respectively, became infected. All 12 controls inoculated with the untreated extract became infected.

The effect of dilution. The infective principle from tomato plants inoculated with the extract from mosaic ground cherry reacts to dilution in the same manner as that taken as typical for tomato, and as that taken from mosaic ground cherries. In a single experiment, inoculations with dilutions of 1 : 10, 1 : 100, 1 : 1,000, and 1 : 10,000 gave 5 out of 6, 5 out of 6, 3 out of 6, and 0 out of 6 infections, respectively. Twelve control plants inoculated with the undiluted extract became infected.

Ability of the infective principle to pass filters. Filtration experiments with the extract from tomato plants inoculated with extract from mosaic ground cherry plants, the results of which appear in table 10, show that the infective entity is capable of passing Berkfeld V and Chamberland F and B filters. The fact that infective filtrates were obtained from both Chamberland filters leads the writer to the assumption that the infective principle also passes the Berkfeld W filter, because this is coarser than the Chamber-

land B filter. Data on this point, however, could not be obtained at the time.

TABLE 10.—*Results of inoculating healthy tomato plants with the filtered extract from tomato plants inoculated with the extract from mosaic ground cherry plants*

Date inoculated, 1924	Filter used	No. plants inoculated	No. plants diseased	Date observed, 1924
July 17	Berkfeld W	6	0	July 29
do	do V	6	6	do
do	Chamberland B	6	3	do
do	do F	6	1	do
do	Unfiltered extract (Control)	12	12	do

PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACT FROM GROUND CHERRY PLANTS INOCULATED WITH THE EXTRACT FROM MOSAIC TOMATO PLANTS

Resistance to ageing. The extract from ground cherry plants infected with the extract from mosaic tomato plants shows a similarity to the typical virus of tomato and of ground cherry mosaic in its ability to retain its power of infecting young ground cherry plants a considerable time after extraction. After 84 days infection was obtained from inoculation with an unpreserved extract. Three lots of six plants each were inoculated with extracts 70, 75, and 84 days old, and 0, 3, and 2 infections resulted, respectively.

Resistance to drying. No experiments were made on the ability of the infective principle in the juice of mosaic ground cherry plants inoculated with the extract from mosaic tomato plants to withstand drying, on account of the fact that the similarity of the viruses of the two plants rendered a difference improbable, especially in view of the results from other tests on this extract; namely, age, resistance to alcohol, and relation to filters.

Resistance to heating. No thermal death point experiments were made with the extract from ground cherry plants inoculated with the extract from mosaic tomato plants since there is no difference in the virus of the two hosts and it was thought likely that there would not be sufficient change to make comparisons possible.

Resistance to treatment with alcohol. The infective entity in the juice of ground cherry plants inoculated with the extract from mosaic tomato plants is as resistant to high percentages of alcohol as the juice of ground cherry or tomato plants infected with the extract from mosaic plants of their own kind. In two series of six plants each, inoculated with the extract treated with 90 and 95 per cent alcohol for one hour, 2 plants in each

series became mosaic. Three of the 6 control plants inoculated with the untreated extract also became mosaic.

Effect of dilution. No dilution experiments were made with the juice of ground cherry plants inoculated with the extract from mosaic tomato plants, since it was not thought likely that there would be a sufficient difference in this respect from the extract from tomato and ground cherry plants inoculated with the extract from mosaic plants of their own kind to make a comparison possible.

Ability of the infective principle to pass filters. Filtration of the juice of mosaic ground cherry plants inoculated with the extract from mosaic tomato plants showed the infective particles capable of passing Berkfeld (grades W. N. and V) and Chamberland (grades F and B) filters. Table 11 shows the results of inoculations made with the filtrate from these different filters.

TABLE 11.—*Results of inoculating healthy ground cherry plants with the filtered extract from ground cherry plants inoculated with the extract from mosaic tomato plants*

Date inoculated, 1924	Filter used	No. plants inoculated	No. plants diseased	Date observed, 1924
May 22	Berkfeld W	4	4	June 1
do	do N	5	5	do
do	do V	6	5	do
do	Unfiltered extract (Control)	5	4	do
July 4	Berkfeld W	6	4	July 14
do	Chamberland B	6	5	do
do	do F	6	5	do
do	Unfiltered extract (Control)	6	5	do

PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACT FROM GROUND CHERRY PLANTS INOCULATED WITH THE EXTRACT FROM MOSAIC CUCUMBER PLANTS

The differences in the reactions of the extract from mosaic ground cherry and that from mosaic cucumber plants, and the ease with which the disease on one of these hosts might be transmitted to the other, offered better possibilities of comparison than in the case of any of the other cross-inoculations. The most striking results in experiments on the properties of the extracts from cross-inoculated plants are from the cucumber-ground cherry crosses, and the tests on ageing, drying, and heating. The other tests with alcohol, dilution, and filtration relationships have appeared so

nearly the same in all cases that nothing could be learned from comparisons of the results of tests on these points.

Resistance to ageing. Since the virus of cucumber mosaic becomes innocuous within a few hours after extraction, it would appear that the virus from ground cherry plants infected with the extract from mosaic cucumber plants would react in the same manner. This is not the case, however, for, as shown in table 12, the extract from ground cherry plants infected with the extract from mosaic cucumber proved infective after 29 days. From the work of Doolittle, a week exceeds the longest limit the virus of cucumber mosaic remains infective; in the present work 24 hours has been the longest period over which the juices of mosaic cucumber plants retained their power of infection. This fact indicates that the so-called virus of cucumber mosaic readily can acquire the property of the ground cherry virus of withstanding prolonged periods of ageing.

TABLE 12.—*The resistance to ageing of the infective principle in the extract from mosaic ground cherry plants inoculated with the extract from mosaic cucumber plants*

Date inoculated, 1924	Age of virus	No. plants inoculated	No. plants diseased	Date observed, 1924
Aug. 25	1 hour (Control)	6	2	Sept. 20
Aug. 5	24 hours	6	3	Aug. 23
Aug. 26	do	6	3	Sept. 20
Aug. 7	48 hours	6	2	Aug. 23
Aug. 26	do	6	4	Sept. 20
Aug. 30	5 days	6	3	Sept. 20
Aug. 12	7 do	6	3	Aug. 25
Sept. 3	9 do	6	1	Sept. 20
Aug. 25	20 do	6	1	Sept. 20
Sept. 3	29 do	6	2	Sept. 26

Resistance to drying. Although the infective principle in the extract from mosaic cucumber plants is absolutely incapable of withstanding drying in any degree, the extract from ground cherry plants inoculated with the extract from mosaic cucumber plants showed no such susceptibility to drying. After 29 days drying, this extract was still infectious. The results of these experiments, set forth in table 13, show that the infective principle of cucumber mosaic may be radically changed in its resistance to drying by transfer to the ground cherry.

TABLE 13.—*The effect of drying on the infective principle in the extract from ground cherry plants inoculated with the extract from mosaic cucumber plants*

Date inoculated, 1924	Length of drying period	No. plants inoculated	No. plants diseased	Date observed, 1924
Aug. 5	Fresh extract (Control)	6	2	Aug. 23
Aug. 6	24 hours	6	2	Aug. 23
Aug. 26	24 do	6	2	Sept. 20
Aug. 7	48 do	6	1	Aug. 23
Aug. 26	48 do	6	3	Sept. 20
Aug. 30	5 days	6	1	Sept. 20
Aug. 12	7 do	6	3	Aug. 25
Sept. 3	9 do	6	3	Sept. 26
Aug. 25	20 do	6	2	Sept. 20
Sept. 3	29 do	6	3	Sept. 20

Resistance to heating. Whereas the extract from mosaic cucumber plants is incapable of withstanding temperatures higher than 70° C., the same virus when transferred to ground cherry plants becomes capable of withstanding temperatures between 85° and 90° C. Three lots of 6 plants each were inoculated with extract treated for 10 minutes at 70°, 80°, and 90° C.; 2, 1, and 1 plants, respectively, became infected. Two of 6 control plants inoculated with untreated juice became diseased.

Resistance to treatment with alcohol. No experiments were made on the resistance of the infective principle in the sap of ground cherry plants infected with the extract from mosaic cucumber plants to treatment with different percentages of alcohol. The difficulty of discerning comparable differences in the reactions of the various viruses by means of this test has made experiments with it of doubtful value.

The effect of dilution. Dilutions of the sap of mosaic ground cherry plants inoculated with the extract from mosaic cucumber plants gave results practically identical with those obtained from dilutions of the viruses from other sources, and further show the difficulty of satisfactory comparisons of different viruses by this method. A dilution of 1:1,000 was found to be the highest dilution possible without reducing the infective powers of the extract. The results of the inoculations with dilutions of 1:10, 1:100, 1:1,000, and 1:10,000 in four lots of six plants each were 3, 1, 2, and 0 infections, respectively. Four of 6 control plants inoculated with the undiluted extract became mosaic.

PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACT FROM CUCUMBER PLANTS INOCULATED WITH THE EXTRACT FROM MOSAIC GROUND CHERRY PLANTS

A lack of time, together with the indications that the difficulty of working with a cucumber extract might be repeated in these experiments, led to the omission of several of the tests used with the other extracts. This seemed especially probable after it was found that the extracts from cross-inoculated cucumber plants showed no greater resistance to ageing and drying than those from cases of ordinary cucumber mosaic.

Resistance to ageing. The fact that the virus from mosaic ground cherry plants is capable of remaining infective for considerable periods of time after extraction does not render the extract from cucumber plants inoculated with the extract from mosaic ground cherry plants any more capable of withstanding ageing than the extract from cucumber plants infected with mosaic from any other source. This is shown in an experiment in which 3 lots of 6 young cucumber plants each were inoculated with extracts 1, 2, and 14 days old. No infections occurred following inoculation with either the 1- or 14-day-old extract, but one infection occurred following inoculation with the 2-day-old extract, a period well within the maximum age limit of cucumber virus as determined by Doolittle. A series of 10 control plants inoculated with extract 2 hours old showed only two cases of infection. This would indicate that even 2 hours' exposure attenuated the infectious nature of the virus.

Resistance to drying. Drying experiments with the extract from cucumber plants infected with the extract from mosaic ground cherry plants have shown that the ability of the ground cherry virus to remain infective after drying cannot be transmitted to the cucumber. In an experiment bearing on this point no infection occurred in any of the plants inoculated in lots of six with the extracts which had been dried for 1, 2, and 14 days. Two out of ten controls inoculated with the fresh extract became mosaic.

PROPERTIES OF THE INFECTIVE PRINCIPLE IN THE EXTRACT FROM MOSAIC CUCUMBER PLANTS INOCULATED WITH THE EXTRACT FROM MOSAIC TOMATO PLANTS BY MEANS OF GROUND CHERRY PLANTS AS INTERMEDIATE HOSTS

It is possible that the results of these experiments can be interpreted merely as additional experiments with the juice of mosaic cucumber plants infected with the extract from mosaic ground cherry plants, but, should the original hypothesis of a specificity of the virus be adhered to, we have in these experiments been dealing with cucumber plants infected with tomato mosaic, even though the tomato virus first passed through the ground cherry.

The explanation given under the preceding section may be repeated here with the addition of a statement of the difficulty in making inoculations on a scale large enough for juice extraction, after passing the virus through an intermediate host plant. This was a time consuming task, and, since the extracts showed the same reactions to ageing and drying as those of typical mosaic of cucumber, the work was limited to these two tests.

Resistance to ageing. Although the viruses of both tomato and ground cherry mosaic are long-lived, the extract from cucumber plants inoculated with the extract from mosaic tomato plants by using ground cherry plants as intermediate hosts did not show a similar property. In four tests, in each of which 6 plants were inoculated with extract 1, 2, 6, and 14 days old, no infection was secured, although the 8 check plants inoculated with 1-hour-old extract gave 3 mosaic plants.

Resistance to drying. It was found that the infective entity in the juice of mosaic cucumber plants inoculated with tomato mosaic by using ground cherry plants as intermediate hosts was no more resistant to drying than the juice of cucumber plants either naturally infected or inoculated with the extract from mosaic cucumber plants. This is shown by the fact that in four tests, in each of which 6 plants (in one case 7) were inoculated with extract after 1, 2, 6, and 14 days' drying no infection was secured, although 3 of the 8 control plants inoculated with 1-hour-old extract became mosaic.

GENERAL DISCUSSION

The results of the present work have shown that the infective agencies in the juices of mosaic tomato and ground cherry plants behave in practically the same manner and are also apparently identical in character with the virus of tobacco mosaic in respect to ageing, drying, heat, dilution, treatment with alcohol, and filtration. The expressed juices of all these hosts are extremely resistant to such treatments and are in marked contrast to the extracts from mosaic cucumber plants in which the virus rapidly loses its infective power after extraction. A summary of the results of tests on the various viruses is given in table 14.

It is also known, however, that tomato mosaic may be transmitted to cucumber by means of the ground cherry as an intermediate host and that cucumber mosaic may be transmitted to tomato in the same manner. Since the symptoms produced by such inoculations seemed the same as those associated with what are considered the typical mosaics of the respective hosts, it seemed possible that the causal agencies of tomato and cucumber mosaic might be the same.

Such an interpretation of the results of these cross-inoculations, however, is open to the objection that the expressed juices of tomatoes and cucumbers show radically different properties, and on this basis it might

TABLE 14.—*Summary of experiments showing the effects of various treatments on the infectivity of mosaic viruses from different sources*

Virus or extract	Infectious after					
	Ageing	Drying	Heating	Diluting	Treatment with alcohol	Filtration
Tobacco	3 yrs.	2 yrs.	85°–90° C.	1:1,000	½ hr. in 80 per cent	Passes Berkfeld and Chamberland
Tomato	138 days	30 days	85°–90° C.	1:1,000	1 hr. in 95 per cent	Passes Berkfeld
Ground cherry	69 days	23 days	85°–90° C.	1:1,000	1 hr. in 95 per cent	Passes Berkfeld
Cucumber	24–48 hrs.	Less than 24 hrs.	70° C.	1:1,000	Negative	Passes Berkfeld
Tomato inoculated from ground cherry	40 days	14 days	85°–90° C.	1:1,000	1 hr. in 95 per cent	Passes Berkfeld and Chamberland
Ground cherry inoculated from tomato	84 days	No test	No test	No test	1 hr. in 95 per cent	Passes Berkfeld and Chamberland
Ground cherry inoculated from cucumber	29 days	29 days	85°–90° C.	1:1,000	No test	No test
Cucumber inoculated from ground cherry	2 days	Negative	No test	No test	No test	No test
Cucumber from tomato through ground cherry	Negative	Negative	No test	No test	No test	No test

seem probable that we are dealing with two distinct causal entities possessing the same host range. The results of the present investigations, however, have shown that the reactions of the infective principle in the juices of mosaic cucumber, tomatoes, or ground cherries always have been the same for a given species, regardless of the source of infection. The extracts from ground cherries infected with the extract from mosaic tomatoes exhibit the same reactions as those from ground cherries inoculated with the extract from mosaic cucumbers, and the same results have held true in the case of cucumber and tomato when inoculated with the extract from either of the other two hosts studied. In short, the so-called properties of the mosaic virus seem to be determined by the character of the juices of the host plant in which the disease occurs.

This variable nature of the infective principle makes it evident that the use of the term "virus" to connote the expressed juices from mosaic plants is misleading, and makes it more necessary that the actual infective entity be isolated from these expressed juices before determining the actual properties and characters of the virus, or infective principle, since by our present methods the tests used indicate changes in the medium rather than in the virus. The results of these studies, however, together with the results of the cross-inoculation experiments of the writer and others seem to indicate a single causal agency in the case of the mosaic diseases of the cucumber, ground cherry, and tomato in so far as their response to a series of arbitrary tests is concerned.

An interesting point in connection with experiments in which the extracts were heated to different temperatures was the relationship between the thermal death point of the viruses and the point at which coagulation occurred. In the case of tomato and ground cherry extracts, the thermal death point was in the neighborhood of 85° C., and it was near this point that complete coagulation took place. In the ground cherry extract two distinct points of coagulation were noticed. The first coagulation separated the green chlorophyllous material from the extract, leaving a grayish substance behind, and occurred in the neighborhood of 65° C. The next coagulation precipitated the grayish substance in curdy masses, separating it from a clear amber liquid. The temperature at which this coagulation occurred coincided with the thermal death point of the virus. This relationship was also noticed in the case of the cucumber, the point of coagulation and the thermal death point both of which are practically the same, 70° C. In experiments in which alcohol was added to the extract from mosaic plants, coagulation also occurred, but there was apparently no correlation between the point in the addition of alcohol at which coagulation occurred and the one at which the virus was rendered innocuous, which in the case of alcohol treatment was not definitely determined. By a combination of these two methods of coagulation, heating and addition of alcohol, under delicately controlled conditions and with suitable filtration methods, a separation of the mosaic extracts into several parts seems possible; one of these parts will be closer to the actual virus than anything heretofore obtained.

SUMMARY

1. This paper presents the results of experiments to determine the properties of the infective principle, or virus, from typical cases of mosaic on the cucumber, tomato, and cultivated ground cherry; and to determine the changes that occur in any one of these viruses when transferred by cross-inoculations to either of the other two hosts.

2. These experiments included studies on the reactions of the various infective principles to ageing, drying, heat, alcohol, dilution, and filtration. A summary of the reactions of the various extracts to these tests is given in table 14.

3. The behavior of the expressed juices of mosaic tomato plants appears to be the same as that described by other workers in the case of tobacco mosaic. The tomato mosaic virus withstands ageing, and can be diluted to 1:10,000 and still produce infection. Treatment for one hour with alcohols ranging in strength from 33 to 95 per cent did not destroy its power of infection. The thermal death point in the case of tomato mosaic appears to lie between 85° and 90° C. The infective principle is also capable of passing all grades of Berkfeld filters.

4. Juices extracted from mosaic ground cherry plants reacted in the same manner as the juices from mosaic tomato plants in tests on ageing, drying, heating, dilution, and treatment with alcohol. Filtrates from Berkfeld filters were also infective.

5. The infective principle in the expressed juices from mosaic cucumber plants is incapable of withstanding ageing, drying, heating, and treatment with alcohol in any degree comparable to that of the virus of the mosaic diseases of tomato and ground cherry. The cucumber virus reacts in the same manner to dilution and filtration through Berkfeld filters as the viruses from the other three plants named.

6. The reactions of the virus in the juice expressed from tomato plants inoculated with the extract from mosaic ground cherry plants were the same as those from tomato plants inoculated with the extract from mosaic tomato plants.

7. The reactions of the infective principle from ground cherry plants inoculated with the extract from mosaic tomato plants were the same as those of the virus from ground cherry plants inoculated with the extract from mosaic plants of their own kind.

8. The juice from ground cherry plants inoculated with the extract from mosaic cucumber plants showed the same reactions as the juice from ground cherry plants inoculated with the extract from mosaic ground cherries.

9. The properties of the extract of cucumber plants inoculated with the extract from mosaic ground cherries were the same as those of other mosaic cucumber plants either naturally infected or inoculated with extract from other cucumber plants.

10. The juices extracted from mosaic cucumber plants infected with the extract from mosaic tomato plants by means of ground cherry as an intermediate host showed no differences from the typical cucumber virus.

11. These experiments have shown that the properties of the mosaic virus of a given plant may be decidedly changed by transferring it to another host. The properties of the viruses from mosaic plants of a certain species also appear to be the same, no matter what the source of infection. This fact indicates that there may be a single causal agent for all the mosaic diseases studied here.

12. Experiments in which the extracts from mosaic plants were treated at different temperatures showed that the thermal death point of the infective principle in the extract and the temperature at which coagulation of the extract occurs are practically the same.

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RESISTANCE OF PEAS TO ROOTROT [1]

FRED REUEL JONES

INTRODUCTION

In the study of diseases of the root system of the pea plant, with which the writer has been concerned for several years, no phase of the general study has proven more elusive of precise statement than that of resistance in certain varieties. The fact that certain varieties were more or less resistant had been observed on the trial grounds maintained by seed companies before it became a matter of investigation and was reported in phytopathological literature. In the several demonstrations of resistance which have been observed by the writer, more than one fungous parasite of the pea plant has been found; but in all cases previous to 1925 the parasite which seemed to be most abundantly present and most aggressive in its attack upon the plant was *Aphanomyces euteiches* Drechsler, described in a previous paper as the cause of pea rootrot. In consequence of this condition, it has been assumed that resistance shown by the plant as a whole was resistance to the destructive action of this parasite or perhaps to some of the consequences of its invasion. In a previous paper[2] summarizing work done up to the spring of 1924, several ways are suggested whereby the resistant varieties may escape injury. Among the more important appeared to be delayed infection, slower progress of the parasite through the root cortex; formation of a secondary periderm, and especially resistance of the vascular cylinder of the root to invasion by soil fungi after the primary cortex has been destroyed. The present paper records the progress which has been made in the study of resistance of peas in 1924 and 1925.

FIELD TRIALS IN 1924

The varieties chosen for closest study in 1924 are given in table 1.

[1] This study of resistance in peas to disease is a portion of an investigation of diseases of peas conducted by the Office of Cotton, Truck and Forage Crop Disease Investigations of the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., in cooperation with the Wisconsin Agricultural Experiment Station.

[2] Jones, Fred Reuel, and Charles Drechsler. Root rot of peas in the United States caused by *Aphanomyces euteiches* (n. sp.). Jour. Agr. Res. 30: 293–325. 1925.

TABLE 1.—*Varieties of peas studied in 1924*

Relative earliness	Varieties	
	Resistant	Susceptible
Early	Rice No. 330	Alaska
Medium	Horal	Horsford
Late	Black Eyed Marrowfat	White Marrowfat

Planting was made at three locations on infested soil where peas had failed the previous year. At Madison, Wisconsin, planting was made by the writer; at McMillan, Michigan, by Dr. Wilber Brotherton, Jr.; and at Columbus, Wisconsin, the first four varieties were planted by the Columbus Canning Company[3] on a commercial scale for demonstration. Unfortunately, the Madison planting was greatly damaged by a heavy shower and was of little value. The soil at Columbus was so hard during an early period of dry weather that roots could not be removed and examined as extensively as was desired. Soil conditions at McMillan, Michigan, were favorable for the removal of roots at all times. In all of these plantings rootrot caused by *Aphanomyces* was the first disease of general occurrence observed, and it developed abundantly and apparently uniformly.

In view of the outcome of this work, it seems unnecessary to report in detail the results of these comparisons. Suffice it to say that, in the early growth of the plants, considerable differences in rate of root growth was found in each location between the varieties, but the differences were not maintained consistently in the three locations.

Apparent delayed infection and retarded progress of the parasite. Two of the varieties, Horal and Black Eyed Marrowfat, seemed to resist infection by *Aphanomyces* in two of the trials. At Madison, a portion of a row of Black Eyed Marrowfats on June 25 yielded 27 plants, seven of which were free from visible infection, while a corresponding row of White Marrowfats yielded only 19 plants, every one of which was thoroughly stripped of root cortex. The roots of the 20 infected plants were not nearly so extensively invaded as were those of the susceptible variety. A similar examination of plants at McMillan planted May 2 and washed from the soil July 11 gave results shown in table 2.

From table 2 it is clearly seen that, although the number of plants examined is small, Horal shows hardly half as large a percentage of infection

[3] The field studies of resistance recorded here have been made possible by Dr. Wilber Brotherton, Jr., who has maintained plots for the writer at McMillan, Michigan, and by The Columbus Canning Company, Columbus, Wisconsin, which has maintained comparative trials of varieties in infested fields for two years.

as the susceptible varieties, and Black Eyed Marrowfat hardly more than a third. These differences in percentage are inadequate to express the entire difference so far as apparent injury was concerned. The diseased plants of a resistant variety were in no case as extensively invaded as were those of the susceptible varieties, a difference that is not readily determined by measurement. When the next examination of the root systems was made on July 31, there were few root systems of any varieties left uninvaded by *Aphanomyces*. The resistant varieties, except No. 330, showed the greater number of infections, but the count of uninvaded plants at this time expresses even less completely the difference in the general condition of the root systems. Between Alaska and No. 330 there was little difference in apparent root injury. The only Horsford plants showing many living roots were a few whose taproots had been destroyed early and which had developed a new root system in the surface soil from the base of the epicotyl. Even the most damaged root systems of Horal plants showed a considerable number of living tertiary rootlets growing from secondary roots deprived of cortex. The same contrast appeared between Black Eyed and White Marrowfats.

TABLE 2.—*Percentage of plants of six varieties of peas invaded by Aphanomyces. Planted at McMillan, Michigan, May 2, 1924. Examined July 11*

Variety	No. plants with rotted root systems	No. plants free from rootrot	Percentage diseased plants
Alaska	39	9	81.0
No. 330	21	3	87.0
Horsford	29	11	72.5
Horal	24	29	45.0
White Marrowfat	57	1	97.7
Black Eyed Marrowfat	18	45	28.5

In contrast with the preceding observations, it must be recorded that at Columbus, Wisconsin, the roots of the Horal pea were invaded just as rapidly by *Aphanomyces* as were those of the susceptible Horsford, but that they did not die as rapidly after being stripped of primary cortex, and that resistance was just as striking here as in the other trials.

Secondary periderm. Comparison of rate and extent of development of the secondary periderm that might protect roots deprived of cortex was made chiefly at McMillan. No difference was observed in behavior between Alaska and No. 330. Periderm development was very slight and incomplete in the taproots of both varieties. Horal showed a greater develop-

ment of periderm than Horsford, and Black Eyed Marrowfat than White Marrowfat; but periderm development was so tardy and incomplete that it seemed unlikely that it contributed greatly toward resistance.

Secondary invasion. In all of the comparisons of susceptible and resistant varieties which have been made in the three locations, one difference has always been found between them. The vascular cylinder of resistant varieties remains free from secondary fungous invasion long after the cortex has been destroyed. For example, in a comparison of Horsford with Horal showing apparently equal injury judged from external appearance of the root systems, razor sections show that, while in Horal only the primary cortex is invaded, in Horsford fungous mycelium is found in some of the larger vessels, and often much of the secondary phloem is disorganized and decayed. All this occurs before the plants above ground have registered any indication of disease. In all varieties, the epicotyl and upper portion of the taproot appear to be the regions where secondary invasion progresses most rapidly and does greatest damage. In resistant varieties, vascular elements, when penetrated by fungous mycelium, usually become filled with a yellow gummy substance which seems to check the invasion, while such yellow discoloration does not appear in the susceptible varieties, where fungous mycelium appears to pass readily from cell to cell. From these examinations it appears that all of the resistance in No. 330 and much of the resistance in the other varieties examined is likely to be found in resistance of the vascular cylinder of the root to invasion and destruction by fungi which enter after the cortex has been destroyed by *Aphanomyces*.

GREENHOUSE STUDY OF DELAYED INFECTION AND RETARDED PROGRESS OF THE PARASITE

In the winter of 1924, efforts were made to demonstrate in the greenhouse ability of resistant varieties to delay infection or the progress of *Aphanomyces* through the roots. In the first trial, peas were planted in boxes 8 inches deep with screen wire bottoms. These boxes were set on moist sand. When the taproots of the young plants had entered the sand, the boxes were lifted and the sand flooded with a suspension of zoospores of *Aphanomyces*. After a time the plants were washed from the soil, and the distance to which the taproots showed softening from decay was measured. The measurements obtained in one trial are given in table 3. In this trial the fungus has progressed a little further through the susceptible variety than through its corresponding resistant variety, but the difference is not large.

In another series of experiments two wire-bottomed boxes of soil 4 inches deep, separated by a space of two inches, were made to fit closely in the

rectangular metal cans used in Wisconsin soil temperature tanks. Peas were planted in the upper box, and the taproots passed through the space separating the two boxes into the lower box of soil. Inoculation was made by pouring suspensions of zoospores on the surface of the soil in the lower box. By this device the time of infection could be easily observed by the discoloration and softening of root cortex at the surface of the soil.

TABLE 3.—*Comparison of rate of progress of the fungus Aphanomyces euteiches through the cortex of roots of peas under identical conditions in each of two trials*

Variety	First trial 9 days at about 20° C.		Second trial 12 days at 20–21° C.	
	No. plants measured	Average distance of invasion (cm.)	No. plants measured	Average distance of invasion (cm.)
Alaska	37	7.0	12	10.00
Rice's No. 330	16	6.0	10	7.90
Horsford	31	7.2	5	10.60
Horal	23	6.6	6	11.20
Black Eyed Marrowfat	13	6.7	10	10.60
White Marrowfat	23	8.1	8	11.30
Yellow Admiral			10	9.55
Green Admiral			7	8.00

Some difficulty was encountered in the use of this device, since the roots passing through the space between the two soil layers were sometimes readily killed by molds which gained entrance, even though the entire apparatus was sterilized before the peas were planted. The root ends were also readily damaged by drying if the upper soil became at all dry. However, in several trials roots in healthy condition were inoculated at the soil surface of the lower box and were observed as the fungus advanced up the taproot into the soil above. In no case was infection of resistant plants delayed beyond that in susceptible plants. There was no conspicuous difference in rate of progress. A typical set of measurements is given in table 3. Here again, with the exception of one pair, there is a difference in favor of the resistant plant, but a difference so slight that it cannot be regarded as significant. Resistance as it occurs in the field cannot be ascribed to such slight retardation of growth of the fungus in the host as is shown here.

FIELD TRIALS IN 1925 AND RESISTANCE TO WILT

Field study of resistance was continued in the spring of 1925. Unfortunately the trial plats at McMillan, Michigan, were discontinued. The

Columbus Canning Company at Columbus, Wisconsin, continued demonstration plats, using a larger number of resistant and susceptible varieties. They were planted on a rootrot infested field on which peas had not been grown for several years. Although there was little rain in early spring, peas germinated and grew fairly well, but rootrot did not appear until late in the season, and then only in small areas. Nevertheless, even though rootrot was absent, susceptible varieties like Horsford and Advancer at Columbus died off almost as completely as in years when rootrot was present, although a little later in the season. Owing to great injury from frost, it was not possible to compare Alaska and No. 330 in a satisfactory manner, but it appeared that Alaska suffered relatively little when compared with previous trials. In addition to the resistant varieties already considered, the Green Admiral pea showed high resistance, if not immunity.

The dying plants of susceptible varieties showed the characteristic symptoms of the wilt disease found by Linford[4] the previous summer, and from these plants the characteristic fungus associated with wilt was repeatedly isolated. It appears, then, that resistance shown by peas at Columbus in 1925 was resistance to wilt. Furthermore, it is probable that wilt has been present at Columbus and perhaps in other experimental plats in previous years, but was obscured by the earlier development of rootrot under usual conditions of rainfall, and that, in consequence, resistance to wilt was one of several factors in the resistance to parasitic invasion, all of which was ascribed to *Aphanomyces*. The frequency and importance of this factor in resistance in past experiments cannot be estimated. It does not seem likely to the writer that this factor has been present in all of the demonstrations of resistance that have been studied. Unless peas can, upon occasion in the field, develop greater resistance to *Aphanomyces* than was demonstrated in the greenhouse trials here described, it appears that resistance to *Aphanomyces* alone is but a small factor in this total of resistance; but it is still possible that the vascular cylinder of some varieties is resistant to destruction by soil fungi after the cortex has been destroyed, a form of resistance that may be coincident with resistance to wilt, or that in certain instances unrecognized root destroying fungi to which some varieties are resistant have occurred in conjunction with *Aphanomyces* in field trials. At present it appears that investigation in these several directions promises to lead to a comprehensive definition of the nature of resistance of peas to soil infesting fungi.

[4] Jones, Fred Reuel, and M. B. Linford. Pea disease survey in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 64. 1–31. 1925.

SUMMARY

1. Resistance to injury from rootrot has been shown consistently by several varieties of peas observed in trial plats at McMillan, Michigan, and Madison, Wisconsin, during several years. Three varieties showing high resistance and three varieties showing conspicuous susceptibility were selected for comparative study in field and greenhouse to determine the difference in behavior of these varieties toward *Aphanomyces euteiches*, which appeared to be the most important cause of disease.

2. In field trials in 1924 one of the resistant varieties showed no delayed infection by *Aphanomyces* nor slower progress of this parasite in destruction of cortex of roots, while two varieties showed conspicuous resistance of this character in the trial at McMillan, but less or none of such resistance in the trials at Madison and Columbus, Wisconsin, where apparent resistance, judged by growth of the plants, was as great.

3. Resistant varieties in all locations remained comparatively free from fungous invasion in the vascular cylinder of root and epicotyl after the cortex was destroyed by *Aphanomyces*, while susceptible varieties were quickly invaded.

4. Greenhouse trials have failed to demonstrate an important degree of resistance to *Aphanomyces* in roots of resistant varieties.

5. In a field trial at Columbus, Wisconsin, in 1925, rootrot was largely prevented by drought. Susceptible varieties were damaged or destroyed by a wilt disease, while resistant varieties remained free from apparent injury.

6. From this experience it appears that resistance, as it is observed in the field, is the sum of several factors. Resistance to rootrot caused by *Aphanomyces* appears to be a very small factor. Resistance to wilt is in some localities a large factor. Other apparent factors whose importance has not been determined are resistance to secondary invasion of roots injured by *Aphanomyces*, and resistance to other root destroying fungi of less conspicuous importance in the soil.

MADISON, WISCONSIN

THE IMPORTANCE OF CONTROLLING CELERY BLIGHT IN THE SEEDBED

A. G. NEWHALL

Observations and experiments during the past four years in the celery fields of western New York have brought out quite strikingly (a) the importance of the seedbed as the source of inoculum both for late blight caused by *Septoria apii* and bacterial blight or leaf spot caused by *Pseudomonas apii*, and (b) the benefit resulting from protecting the seedlings with a copper fungicide.

Many authors have pointed out that certain seed-borne and soil-harbored pathogenes attack their hosts before they are out of the seedling stage. Several investigators have recommended more recently that celery spraying be commenced in the seedbed (3, 5, 6). There is some evidence that good control of tobacco wildfire may be obtained by this practice (1, 2). Data are presented here which indicate that delaying primary infections for 5 or 6 weeks in the case of celery blights by dusting the seedlings may be reflected later in the season by substantial reduction in blight development and at harvest by a corresponding increase in yield.

It has been recognized for some time that the late blight fungus may live over in the soil or on the seed and is often carried with the seed. The method of overwintering of the bacterial blight pathogene, on the other hand, is not so well understood. It is not likely that seed transmission is the common method, for this disease is rare, if present at all, in the celery seed growing districts. Moreover, in the muckland regions of New York State, where the disease is common, it is much more prevalent in fields where celery follows celery repeatedly than where a two-year rotation is practiced. The important fact at present is that, in certain seasons characterized by frequent or prolonged rains in June, the blight pathogenes become established on celery plants while they are still very small and before they are removed from the seedbed. Primary lesions both of late blight and bacterial blight have been found repeatedly under these conditions on plants only 5 or 6 weeks old.

The value of growing the plants in a clean seedbed was indicated by the following observations. On one farm plants for the field were selected from

TABLE 1.—*Field counts of blight*[a] *on plants from dusted and untreated seedbeds, 1923 and 1924*

Seedbed location, history and treatment	No. of plants examined	Percentage developing blight	Average no. of spots per plant	No. of dead or yellow leaves per plant	Yield in crates per acre	Percentage increase in yield
a. Old celery muck; seedlings dusted twice	1,000	3.9				
b. Same not dusted	1,000	15.4				
a. Old celery muck; moist situation, dusted 3 times	1,000	1.2			370	16
b. Same not dusted	1,100	26.3			321	
a. Old celery muck; moist, dusted 3 times	2,355	5.9	355			
b. Same not dusted	1,994	27.+	1,154			
a. Old celery muck; moist, dusted 3 times	500	14.2	163			
b. Same not dusted	500	93.0	1,989			
a. Old celery muck; dusted twice	500	29.0	112	1.2	383	36
b. Same not dusted	500	87.0	979	2.8	280	
a. New muck never cropped to celery; no treatment	1,000	6.4	145	.8	420	6.4
b. Old muck; no treatment	1,000	50.6	1,087	3.0	395	

[a] Both late blight, caused by *Septoria apii*, and bacterial blight, caused by *Pseudomonas apii*, present. All others bacterial blight only.

two seedbeds, one located on old muck which had grown blighted crops of celery in previous years, and the other on new muck. Neither seedbed received any fungicidal treatment. Plants from the two seedbeds were transplanted to the same field in July but were set in separate blocks of approximately an acre each. Six weeks later examination of 2,000 plants in this field showed that 50.6 per cent of the plants from the old muck seedbed were spotted, while only 6.4 per cent from the new muck were diseased (table 1). In other words, there were over seven times as many blighted plants from the old muck seedbed.

Apparently few workers have appreciated the number of plants which become infected in the seedbed and which are not detected at transplanting time. To illustrate this point, a seedbed was selected where blight was known to have existed the year before and where a very slight amount of infection was found in two small areas. Any celery grower would have pronounced it a clean seedbed. The day after this examination the plants were set out in the field. Three days later a very careful examination was made of all the plants in 33 rows 65 feet in length, and a stake was placed beside each spotted plant. Out of 7,690 plants examined, 83 were found to be affected by bacterial blight. The area amounted to one-fifth of an acre, which meant that 415 focal points for blight development per acre had come out of this seemingly healthy lot of seedlings. The distribution of these plants is shown in figure 1, a photograph of about one-third of the area examined.

Experiments were initiated in 1923 by the author (4) to determine the value of dusting to protect the seedlings from the time they were half an inch high until they were transplanted to the field. This procedure had already been tried by at least one enterprising celery grower, who had observed less blight all through the season on plants taken from seedbeds which he had sprayed than on plants from untreated seedbeds. On four farms 20–80 copper-lime dust was applied to portions of the seedbeds. A Niagara blower gun or hand duster was used, and from two to four applications were made at approximately seven-day intervals, beginning when the plants had developed their first true leaves. On two of these farms blighted celery had occupied the land the preceding year. Furthermore the moisture supply was exceptionally close to the surface, thus presumably favoring the blight pathogenes. On the other two farms, however, celery had not been grown for several years. Bacterial blight was found in the untreated portions of the beds in abundance on the first two farms, and on one of them Septoria blight was also abundant. But no blight was found on the other farms before the plants were set out in the field. The dusted plants were

set in a three-row block alternating with blocks of undusted plants. All received the same treatment after they were transplanted to the field.

During the last of July, after the plants had become well established, the fields were inspected and a record made of the number of plants showing blight spots (table 1). In one field, out of 1,000 plants which had been dusted twice in the seedbed, 39 plants, or 4 per cent, were spotted. In the same field, out of 1,000 plants from the undusted portion of the seedbed, 154, or 15 per cent, were found spotted. In another field, out of 1,000 plants which had been dusted three times in the seedbed, only 13, or 1.3 per cent, were diseased, while of an equal number from the undusted portion of the seedbed 290, or 29 per cent, showed blight spots. So there were in July from 4 to 22 times as many focal points from which the blight pathogenes could spread in those parts of the fields set with plants which had not been protected in the seedbed. In the last case above, the difference in blight was evident throughout the season even to the casual observer. Both late

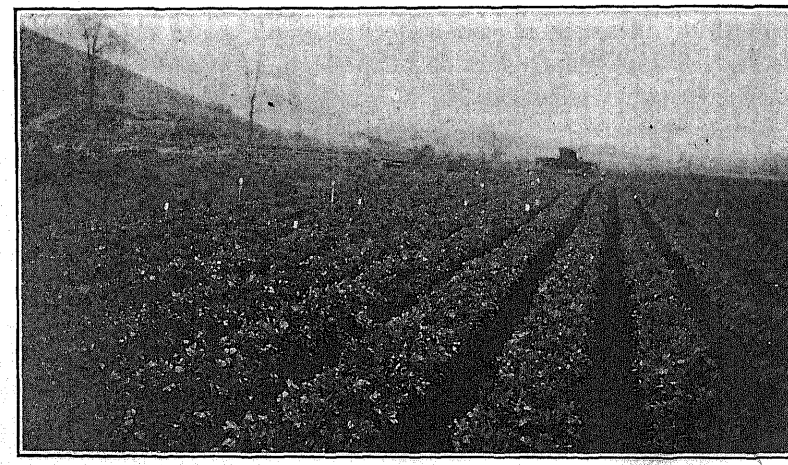

FIG. 1. Stakes show where blighted seedlings had been set; 415 focal points for blight development per acre. Plants were taken from seedbed in continuous celery culture. These had not been sprayed or dusted but looked healthy to the casual observer at transplanting time.

blight and bacterial blight were abundant throughout this field. At harvest time three rows of plants which had been dusted in the seedbed were found to fill 18 crates, weighing a total of 1,950 pounds; while three adjacent check rows filled but 16 crates, weighing 1,750 pounds. This increase of 16 per cent amounted to 49 crates per acre.

In 1924 the experiments were repeated on five other farms. From two to three applications of 20–80 copper lime dust were made to portions of the

seedbeds. As in the previous season, on two of the farms there appeared to be no benefit from dusting the seedlings in the bed. Practically no blight developed on either the dusted or untreated plants until midsummer, when the growers commenced spraying or dusting the fields themselves. This is thought to be due to rotation of the seedbeds and to their well drained locations. On the other three farms, however, as table 1 shows, there were 3, 4½ and 6½ times as many blighted plants by midseason in those portions of the fields set with plants which had not been dusted in the seedbed. If the actual number of spots of blight per plant are considered, there were 3, 8½ and 12 times as many spots where the plants had not been dusted in the seedbed.

In 1924 also, increases in yield similar to those mentioned for 1923 were obtained. For example, on that part of one farm planted from a seedbed which had received two applications of 20–80 copper lime dust and which was relatively free from disease, there was an increase in yield of 103 crates per acre. On another farm where part of the plants came from a seedbed located on new muck and which remained relatively free from blight throughout the season, there was an increase in yield of 25 crates to the acre over that portion of the field set with plants from the old muck seedbed.

It is evident, therefore, that, if the grower can not choose new muck for his celery seedbed, and it is often undesirable to do this on account of soil moisture conditions, it is then highly desirable to protect the young seedlings in the seedbed. From the time the plants put out their first pair of true leaves until they are transplanted, 2 to 4 applications of a copper fungicide should be made at suitable intervals. The frequency of the applications will depend on rainfall and the rate of development of the plants, the aim being to keep them well covered at all times. No injury has been noted even from large quantities of copper-lime dust. It is believed that the fungicide acts not only as a protective coating on the plants but also inhibits or even kills the pathogenes which may be residing close to the surface of the soil. In all events the early applications made before the plants were large enough to completely shade the ground were the more important ones. Bordeaux mixture is known from earlier work (4) to be equally as effective as copper-lime dust in controlling these two blights of celery. However, on account of the convenience of the dusting method, growers who would never have bothered with a hand sprayer are now using a hand duster without complaint and with excellent results. The long quiet evenings in June are ideal for treatment with a minimum expenditure of time and material. The dust settles close to the ground in a dense layer, giving a very complete covering.

A few applications to the seedbed at a very small cost have been found to save one or more applications of fungicide, or of fertilizer, to the entire field later in the season. It is a most reasonable form of insurance against both late and bacterial blights. In fact it is at present probably our only safe means of protection against the seed-borne late blight organism. Seed treatment may cause more or less injury, depending on the age and vitality of the seed; and furthermore the late blight pathogene may live over in the soil, in which case seed treatment would be useless.

SUMMARY

1. Both late blight (caused by *Septoria apii*) and bacterial blight or leaf spot (due to *Pseudomonas apii*) of celery have been found in considerable abundance on small plants in out-of-door seedbeds.

2. When two to four applications of 20–80 copper lime dust were applied while the plants were in the seedling stage, a marked reduction in subsequent blight development throughout the season was obtained in six out of ten fields. The control of blights was reflected in substantial increases in yields at the end of the season.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, N. Y.

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SMUT OF WESTERN RYE GRASS

W. P. Fraser and G. A. Scott

A smut of western rye grass was first brought to the attention of the senior author in 1916 on specimens collected at Saskatoon and transmitted by Professor James Murray of MacDonald College. He reported about 60 per cent infection. More recently this smut has been collected at Winnipeg, Brandon, Indian Head, Scott, Lacombe, and at a number of other places. There was usually about 10 to 15 per cent infection where the collections were made. One district in northern Saskatchewan, Sturgeon River, reported as high as 90 per cent of smut in a field of western rye grass. As this grass is largely used for hay in the prairie provinces of Canada, and as the smut reduces its value for feeding purposes and seriously interferes with the production of seed, a study of the smut seemed desirable.[1]

The smut attacks the spikelets, destroying the ovaries and usually the bases of the glumes (Fig. 1), although frequently the outer glumes are not affected. The sori are large, irregular, rather firm at first, but finally dusty. The spore mass is dark brown to black in color. Usually all the spikelets of the head are affected, but sometimes only the upper ones are smutted. The spores are usually spherical, sometimes oval, dark brown, finely verrucose, and mostly 8–11μ in diameter, averaging 9.2μ. The spores germi-

TABLE 1.—*The effect of treating seed of western rye grass for control of smut*

Year	Place	No. of rows of each treatment	Per cent smut in rows from seed treated in different ways		
			Seed untreated	Seed dusted with smut spores (Check)	Seed dusted with smut spores and treated with formaldehyde solution (1–320)
1919	Indian Head	2	0	15.0	0
do	Saskatoon	2	trace	8.0	0
1920	Indian Head	2	0	12.5	0
do	Saskatoon	2	0	23.3	0
1921	Indian Head	1	trace	80.0	0
do	Saskatoon	1	trace	75.7	0
1922	Saskatoon	1	trace	24.6	0

[1] Fraser, W. P. A smut of western rye grass. Phytopath. 10: 316. 1920.

nated readily in 5 per cent sugar solution and less freely in water. Sporidia are produced in sugar solution in a few hours (Fig. 2).

Experiments carried out during the seasons 1919–1922 indicated that the smut was carried over from season to season by spores clinging to the seed, and that seed treatment with formaldehyde solution would prevent the

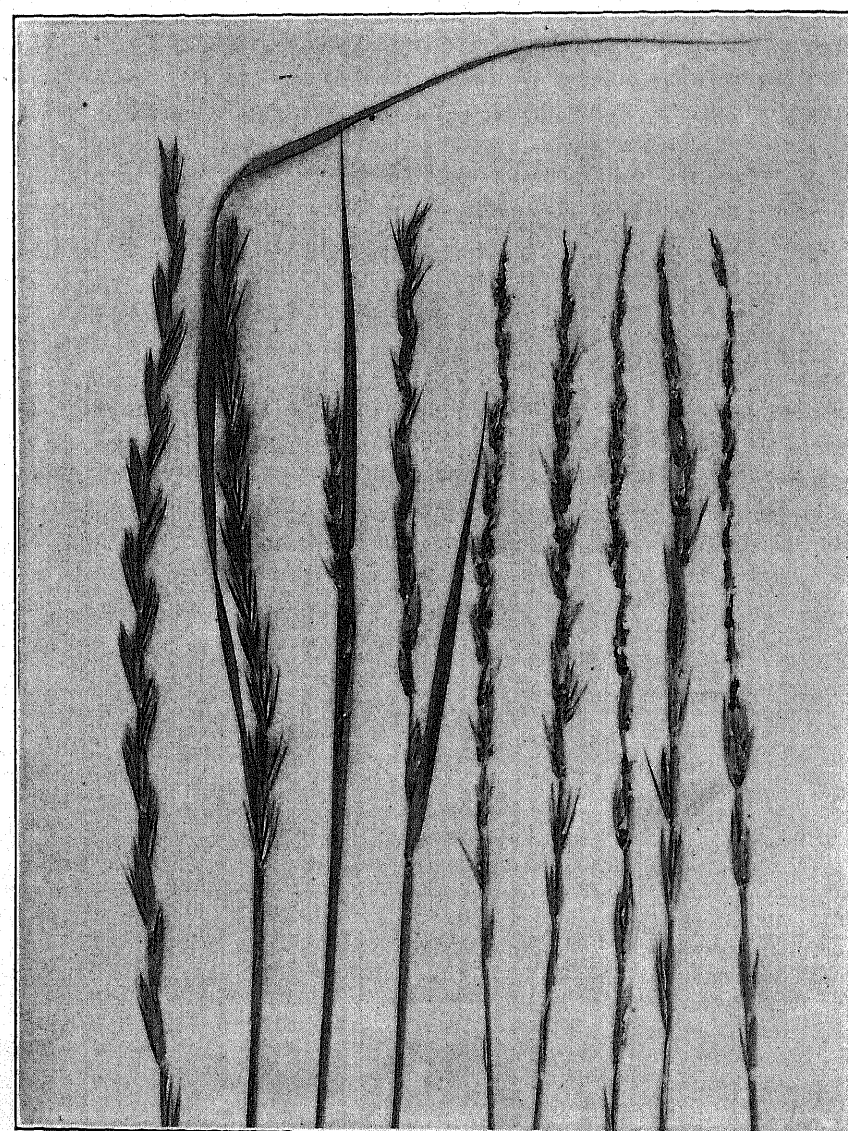

FIG. 1. Healthy and smutted heads of western rye grass.

smut. The experiments were not extensive; only one or two 16-foot rows were grown at each of two stations during four seasons. Seed artificially inoculated with spores was used. The results, however, were marked and consistent throughout, so there seems little doubt that ordinary formaldehyde treatment, 1–320, is effective. There was no evident injury to the seed from this treatment. A summary of the experiments is given in table 1.

Copper carbonate dust was tested in 1922 as a seed disinfectant for the control of this smut but was not effective, since 10 per cent of smut appeared in the treated rows. The checks showed 24.4 per cent of smut.

In 1920 seed of *Agropyron tenerum* was dusted with spores two years old from the same host. The dusted rows averaged 4 per cent of smut at Saskatoon and 3.75 per cent at Indian Head. The check rows were free from smut. Two 16-foot rows were used at Saskatoon and one at Indian Head. This indicates that the spores may retain their viability at least two years.

An experiment to determine the host range was made in 1924 at Saskatoon. Seed of various grasses was dusted with the spores of this smut from *Agropyron tenerum* and seeded in 16-foot rows. The results are given in table 2.

TABLE 2.—*Results of inoculating the seed of different species of Agropyron and Bromus with smut from Agropyron tenerum, 1924*

Row no.	Seed of	Notes	Per cent smut[a]	
			1924	1925
1	*Agropyron tenerum*	commercial seed	5.7	4.0
2	do	seed artificially smutted	47.9	23.2
3	do	do	50.8	20.6
4	*A. richardsoni*	do	14.6	4.1
5	*A. dasystachyum*	failed to head		6.0
6	*Bromus ciliatus*	3 smutted heads. Only a few plants headed		6.0
7	*B. latiglumis*	1 smutted plant. Only a few plants headed		.8

[a] As some of the grasses did not head in 1924, the rows were left until the next season, when all the grasses headed abundantly.

While it is suggested by the experiment that the smut on the Bromes came from the spores applied to the seed, yet, as no check rows were seeded, the experiment does not establish this.

Additional rows of seed treated with smut spores were seeded in Saskatoon in 1925. The results of this test are given in table 3.

Systematic position: The smut is closely related to *Ustilago bromivora*, the spores of which are slightly larger and the surface usually less finely verrucose. Measurements of 100 spores from *Agropyron tenerum* and *A. richardsoni* gave an average diameter of 9.23 and 9.26μ respec-

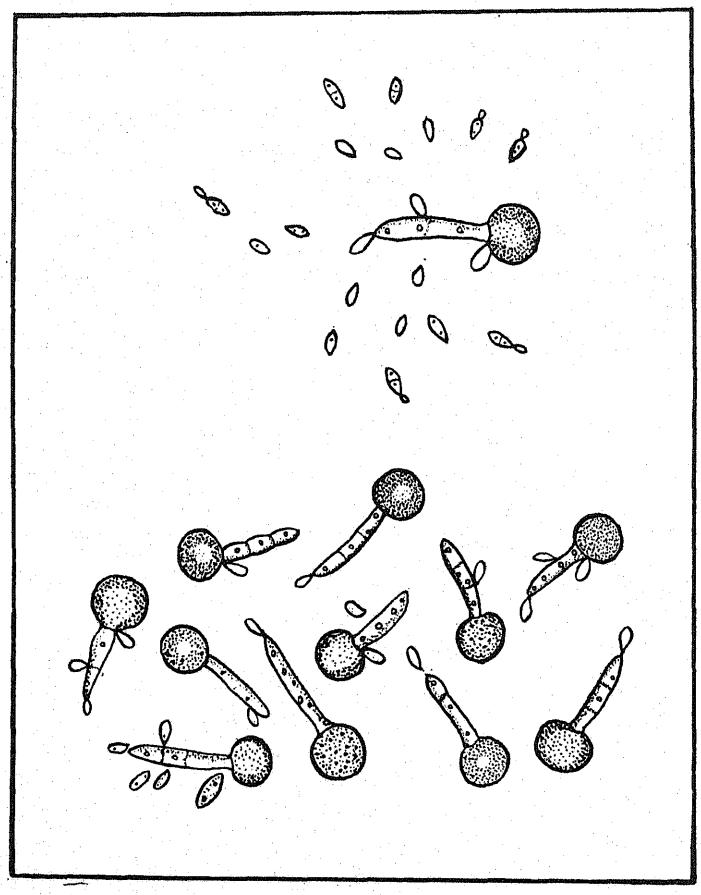

FIG. 2. Germination of spores of *Ustilago bromivora*.

tively, and the same number of spores of *Ustilago bromivora* from *Bromus ciliatus* and *B. tectorum* measured 9.94 and 9.53μ respectively. It seems best to regard this smut on Agropyrons as *Ustilago bromivora*. One field collection was made on *Agropyron richardsoni* at Saskatoon.

TABLE 3.—*Results of inoculating the seed of different species of Agropyron and Bromus in 1925*

Row no.	Seed of	Per cent smut
1	*Agropyron tenerum* (dusted with smut)	19.0
2	do (check)	0
3	*A. richardsoni* (dusted with smut)	16.8
4	do (check)	0
5	*A. dasystachyum* (did not head)	
6	do (do)	
7	*A. spicatum* (did not head)	
8	do (do)	
9	*Bromus ciliatus* (dusted with smut). A few plants headed.	0
10	*B. ciliatus* (check)	0
11	*B. porteri* (dusted with smut). A few plants headed.	0
12	do (check)	0

SUMMARY

A smut of *Agropyron tenerum* is common in the prairie provinces of Canada. It also attacks *A. richardsoni* and *A. dasystachyum*.

The smut closely resembles *Ustilago bromivora* and is assigned to that species.

The smut is controlled by the formaldehyde solution as used for cereals.

The experiments here described were carried out as part of the work of the Dominion Laboratory of Plant Pathology at the University of Saskatchewan. Acknowledgment is made of the assistance of the staff of the Laboratory in these experiments, and of the facilities afforded by the University for carrying on this work.

UNIVERSITY OF SASKATCHEWAN,
SASKATOON, SASK.

OBSERVATIONS ON INFECTIONS OF APPLE AND PRUNE ROOTS BY ARMILLARIA MELLEA VAHL

S. M. Zeller

Although the Armillaria rootrot is one of the oldest known plant diseases, the exact manner in which it enters a host plant is imperfectly known. It is usually taken for granted that spores of this fungus do not directly infect living hosts. Therefore, it must be a positive deduction that infection by the mycelium must take place directly by individual hyphae or by hyphae organized as rhizomorphs. Infection is not known to take place through roots which are too young to have developed a bark. In all reported observations, infection took place through rootstocks or larger roots, but there still seems to be question whether entrance is effected through healthy, uninjured bark, through wounds or through dead roots.

Hartig (4, 5) and Cieslar (3) experimented on a variety of dicotyledonous woody plants and arrived at about the same conclusions as those obtained by Wagner (7). Wagner planted 43 small trees of different sorts in garden soil which was infested by rhizomorphs of *Armillaria mellea.* Of these, 24 were intentionally wounded on the roots. Seven of the wounded trees became infected, while but one of the 19 unwounded was infected. Brefeld (2) found that, when freshly cut pieces of thick roots of Scotch pine were brought into close contact with rhizomorphs, the tips of the latter grew into the roots very quickly, both through the cut ends and through the undamaged bark. However, cut pieces of roots, especially coniferous roots, cannot be considered as healthy, and it is difficult to remove pieces of root without injury to the bark. It should be considered from his experiment not that rhizomorphs can force their way into the bark of a perfectly healthy root but that they can enter dead, or partially dead, bark.

Barss (1) suggested that "rhizomorphic strands . . . upon coming in contact with the root of the susceptible plant, may possibly bore their way into the healthy bark. It is believed by man, however, that the fungus gains its entrance, more often, if not exclusively, at points where the root or the crown of the tree has been injured."

Horne (6), who perhaps has observed Armillaria root rot of orchard trees in more of its various conditions and appearances than any other

American worker, observes that "when the tip of a rhizomorph comes to a healthy root the very small microscopic threads of which it is composed seem to loosen like the cut end of a rope and the individual threads penetrate into the bark and start a new infection." This comes the nearest to describing actual infection of healthy tissues that the writer has found reported.

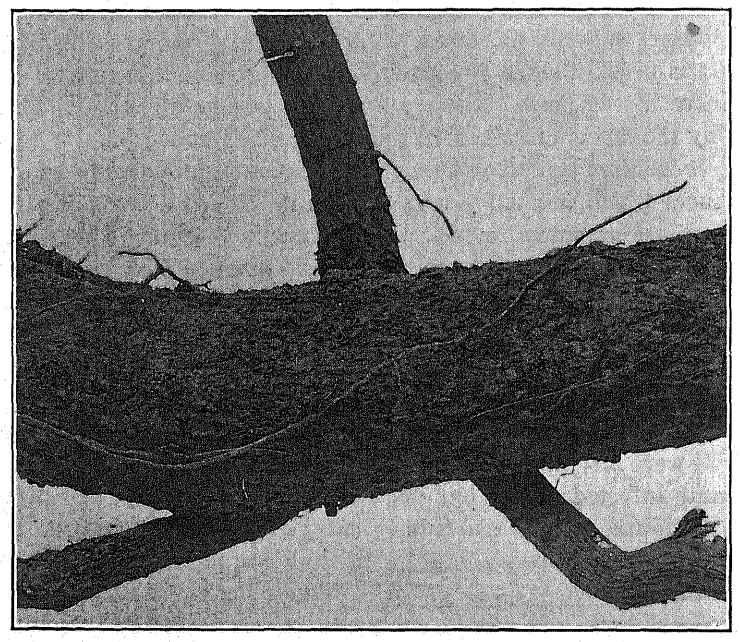

FIG. 1. A 3-year-old apple root which shows an oak (*Quercus garryana*) root thoroughly infected with *Armillaria mellea* lying in contact with the fork of the apple root as they were found *in situ*.

The Armillaria rootrot is a widespread, serious orchard disease in the Northwest, especially in that portion of the coast states west of the Cascade Mountains. The disease is particularly severe on prune trees and is rather serious in many apple orchards. In this region the strain of *Armillaria mellea* Vahl which grows on oak (*Quercus garryana*) is the one which causes destruction on orchard trees.

Since the fall of 1922 the writer has had several opportunities to observe infection of apple and prune roots by this oak-root fungus. Many examples of infection which were apparently through wounds have been found. These were near the collar of the tree not far from the ground line. However, after finding a few cases of infection of apple roots where there were

no signs of previous wounds, attention was attracted to the desirability of making further observations. Shortly, many more examples of this type of tissue invasion were found. One of the largest examples of this type of entrance to the host is illustrated in figures 1 and 2. Figure 1 shows a piece of oak root which was about 1½ inches in diameter and 18 inches long, lying across the fork of a branching apple root. These roots bore this exact relation to each other in the soil and were in contact. Figure 2 shows the same portion of the apple root as shown in figure 1 with the piece of oak root removed. The piece of oak root was thoroughly decayed by *Armillaria mellea* with the exception of a few sectors which were merely sufficient to identify the wood. The most interesting feature of the situation is the scurfy, cankered condition of the apple root where it was in contact with the diseased oak wood. Figure 3 illustrates a more developed stage of the same type of condition in a slightly older apple root. In this instance a diseased apple root crossed this apple root and made contact with it at the groove, designated by the circle. In the example illustrated in figure 1, infection had not gone farther than the inner parenchyma of the bark, while in the case illustrated by figure 3, infection had reached the cambium and had spread up and down the cambium from the point of penetration for a distance of about 2 cm. At the same time the fungus had penetrated the wood to a depth of about 2 mm. and up and down a total length of about 1.5 cm. The infected area of wood had the characteristic odor and color of Armillaria rot and was bounded by the characteristic dark line, while the inner bark was full of white mycelium bounded on the outer borders with a dark brown, fungous crust. The apple root illustrated in figure 1 was three years old when taken and that in figure 3 was four years old. There were no indications of wounds of any sort that might have been present in either case before the infections started. From the contour of the root illustrated in figure 3, it was not difficult to estimate that the two apple roots had been in contact for at least three years. This, however, led to no information as to the length of time necessary for the cambium to become infected, for there was no way to estimate the length of time the thoroughly diseased root had been diseased in that portion directly in contact with the root recently infected. Seventeen cases of this type of infection of apple roots have been observed in various stages of the scurfy bark. Five similar cases have been found on the roots of Italian prune trees.

Undoubtedly many who have had orchard experience with Armillaria rootrot have observed its toxicity to affected trees. Whether this toxicity is due to the presence of the fungus itself or to the products of its metabolic activity in the wood is not known. Especially where this rot has been found affecting prune trees, the writer has seen many cases of the toxicity referred to. If a tree has only one root affected, with infection extending well up

toward the collar, at first only those branches immediately above the affected root will yellow and die, although the whole tree may finally die. It is not the loss of the root which has affected the branches above, for, when such a root is pruned, the branches of the tree apparently go unharmed. It seems that the death of branches in such cases must be brought about by the toxicity of the product of the organism working in the wood.

The writer believes that the type of infection mentioned above has its start through healthy bark which has been acted on by toxic substances given off by tissues decayed by *Armillaria mellea*. The very first indication of this toxic influence on the bark is the appearance of a shallow, browned

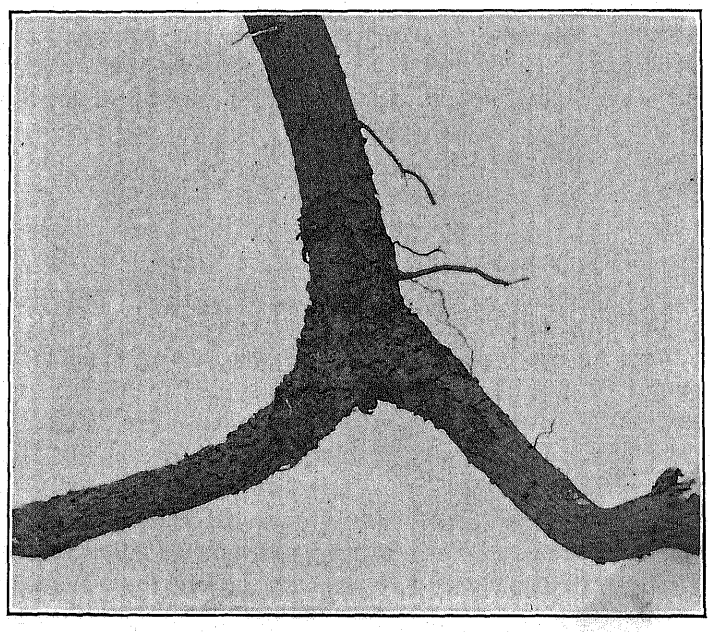

FIG. 2. The same portion of the apple root shown in figure 1 with the oak root removed. Notice the scabby, cankered appearance of the apple bark where it was in contact with the Armillaria-decayed oak root.

spot in the outer parenchyma of the bark of the root just around and under the lenticels. These are at first of a blistery, edematous consistency and later dry down and flake. Soon several such blisters may appear and flake. When dried, the thin circular flakes curl up at the edges, the appearance of the bark reminding one of the rough bark produced by pear scab infections on young twigs. It resembles a type of so-called "burning" which may take place when organic débris is piled too closely to young bark. These spots coalesce until one soon finds a superficial condition like that illustrated in figure 2. Evidently, this condition is extended deeper very

slowly, new layers of dead flakes being sloughed off as new plate-like cork layers are formed in the bark parenchyma. This continues until a cankered condition appears as illustrated in figure 3, when the mycelium of the fungus is able to pass beyond the inner parenchyma of the bark to the cambium. In the earlier stages of the flaking off of the outer parenchyma, no mycelium of the fungus was found in the affected spots until at least two or more plates of cork had been sloughed.

This type of infection is apparently a very slow process, but the wound through which the fungus enters is an indirect result of the presence of the organism or its metabolic products. No attempt has been made to deter-

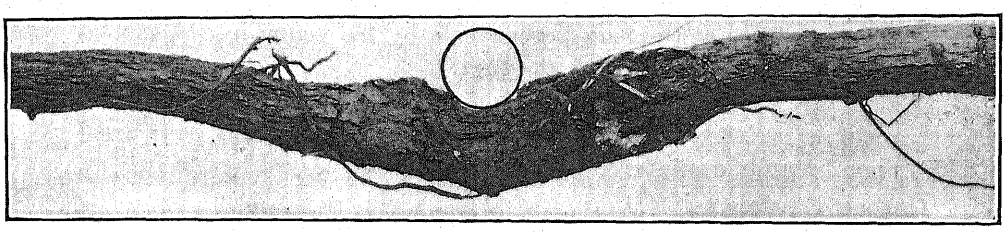

FIG. 3. A 4-year-old apple root showing the cankered condition produced by an Armillaria-decayed apple root which crossed in contact with it in the position of the circle. In this case the Armillaria infection had penetrated to the cambium.

mine the nature of the products which are toxic to the bark tissues.

A few observations have been made in connection with another type of infection of the roots of prune trees. A note concerning the place of entrance and the writer's theory as to the mode of entrance might be an aid to others doing similar field observation. It has been found that parent roots are frequently infected around newly formed lateral roots. Cases have been found where only the parenchyma of the parent root immediately surrounding a new lateral in its first year's growth was invaded by the mycelium of *Armillaria mellea,* and more advanced stages where this has reached the cambium have been observed. The writer has assumed that the rupture of the bark parenchyma by the young lateral root as it pushes out through the bark has provided an entrance in such cases where a vegetative portion of the fungus chanced to be near at the time of rupture. The several instances of this kind have shown nothing to disprove the assumption, although actual penetration has not been followed.

SUMMARY

1. Rootrot, caused by *Armillaria mellea* Vahl, is a widespread, destructive orchard disease in the Northwest, especially in that portion of the coast states west of the Cascade Mountains.
2. Infection of the roots may take place in at least three ways:
   (a) Through wounds. Examples of this type of infection are common.
   (b) At points of contact of diseased and healthy roots. Seventeen cases of this type of infection of apple and five similar cases of prune infection have been observed.
   (c) At point of emergence of lateral roots, apparently through the rupture of the bark parenchyma by the young lateral root as it pushes out through the bark.
3. When infection occurs at the point of contact of diseased and healthy roots, it probably starts through healthy bark which has been acted upon by a toxic substance given off by tissues decayed by the fungus.

OREGON EXPERIMENT STATION,
CORVALLIS, OREGON

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A SPOT-ROT OF APPLES IN STORAGE CAUSED BY BOTRYTIS[1]

F. D. Heald and Roderick Sprague

INTRODUCTION

A storage rot of apples caused by *Botrytis cinerea* has long been known in the Northwest. Briefly, it is a light brown, moderately soft rot which, under moist conditions, develops grey conidial tufts that completely cover the surface of the decayed fruit.

During the fall of 1921 a striking departure from these ordinary symptoms was noticed. On a single Rome Beauty apple from common storage, showing a pale brown lesion, nearly every lenticel in the rotted area was surrounded by a darker brown ring. Except for these peculiar reddish-brown spots which had not heretofore been associated with the rot, the appearance of the host and fungus was characteristic of *Botrytis cinerea.* Microscopic examinations and isolation cultures gave what appeared to be typical *Botrytis cinerea,* but at that time no further study was made of the rot.[2]

In early November, 1923, Rome Beauty apples affected with the spot-rot were obtained from one cellar, and a few days later Delicious and Wagener apples from another cellar at Pullman. It has since been observed in common storage Romes at Spokane. From the time of the first collection of apples in November until the last of February, the spot-rot of apple could be found in the storage cellars around Pullman. Sufficent material was on hand during the season for a more detailed study of the spotting and its cause.

CHARACTERISTICS OF THE SPOT-ROT

Botrytis rot of apples is generally light brown in color. The decayed areas frequently have at first a pale, translucent, watery appearance which soon changes to a duller brown. The striking feature of the rot was the evident darker spots which always centered around the lenticels. They are brownish-red rings of about 2–3 mm. diameter, each showing a pale or

[1] Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 133, College of Agriculture and Experiment Station, Pullman, Washington.

[2] Heald, F. D., and Dana, B. F. Notes on Washington plant diseases. I. Botrytis diseases. Trans. Am. Mic. Soc. 43: 136–144. 1924.

whitish area in the center. This lighter region at the center and a paler zone on the outside cause the rings to show up in a striking fashion. The spots are not numerous, averaging 4–20 to the square inch, and give to the surface an appearance similar to that of a polka-dot fabric (Fig. 1).

After being left in damp chambers in the warm laboratory a few days, the spots lose their definiteness and spread out as blotches or darker discolorations on the surface of the fruit. They do not lose their identity entirely, however, as the white area in the center of the spot remains to mark the place where the spot originated. Conidial tufts soon begin to push through the surface, and in a few days the fruit is a mass of grey mold.

ISOLATIONS

Isolations were made from partly decayed and spotted fruits, previous to the appearance of any external conidiophores, by removing bits of tissue from the advancing edge of the rot with a flamed scalpel and planting them on poured plates. Three per cent dextrose agar + 10 was used. On this medium a flat spreading growth developed, which gave rise in a few days to the grey, fluffy patches of fruiting *Botrytis*. At the same time the cultures showed a peculiarity in the nature of a red coloring of the substratum which was not typical of *Botrytis cinerea* as commonly isolated from apples in this region. Microscopic examination of the cultures showed typical Botrytis spores and conidiophores, agreeing in spore measurements fairly closely with *Botrytis cinerea* ($4.6 - 7.5 \times 7.5 - 13.1\ \mu$).

INOCULATIONS

From the fact that the isolated fungus acted peculiarly on media, and because the symptoms on the host were different from the usual Botrytis rot, inoculations were made to determine whether the common *Botrytis* or any of its strains would cause the spot-rot under laboratory conditions. Before carrying on inoculation experiments, a known pure culture of *Botrytis* was obtained by making a streak plate from spores from tufts on the surface of a Botrytis-rotted fruit. The two following cultures were thus available for use: (a) the "unknown" culture obtained from mycelial transfers from a spotted apple, (b) pure *Botrytis* from spores. The pure *Botrytis* culture did not color the substratum. Rome Beauty and Jonathan apples were inoculated with these two cultures. The inoculations were made by cutting rectangular flaps in the apple with a flamed scalpel and inserting a small mass of mycelium under each flap. Uninoculated checks were kept. The different lots of apples were placed in sterilized damp chambers and kept in the laboratory at room temperature.

The development of the rot was observed daily over a period of two weeks. Spotting occurred along the advancing edge on apples inoculated

with the "unknown" cultures and also faintly on apples inoculated with the pure culture of *Botrytis*. At the temperature at which the apples were held, the experiment was inconclusive.

Next, inoculations were made to show whether the culture of known *Botrytis* would cause spotting simliar to that caused by the original isolations if the specimens were incubated at a lower temperature. Since Jonathan apples had not proved satisfactory, Rome Beauty apples were used exclusively in the following inoculations with:

(a) the original culture from spotted Rome apple;
(b) known *Botrytis* from spores;
(c) rotted tissue direct from spotted Delicious apple.

These were made in duplicate and placed in moist chambers. One set was left in the laboratory at room temperature, and the other was placed

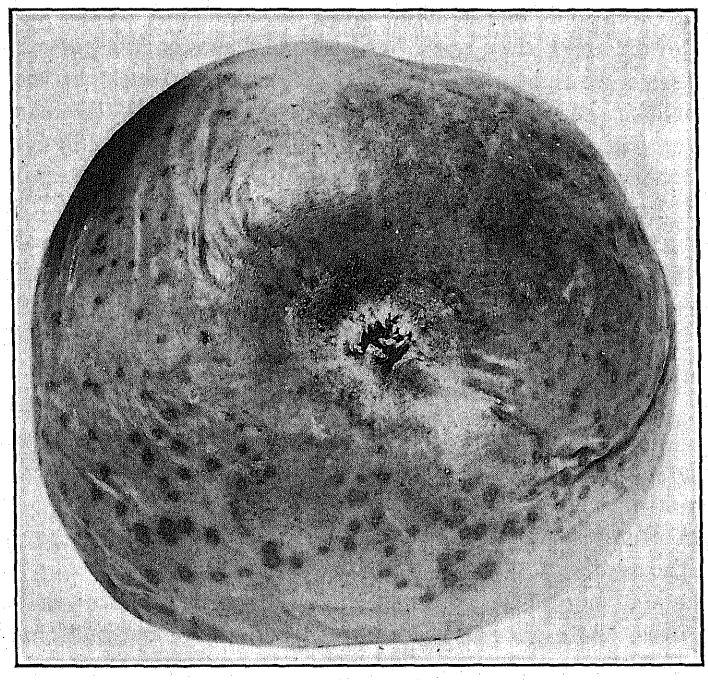

FIG. 1. Rome Beauty apple completely rotted by *Botrytis cinerea*.

in a cool storage cellar at 35°–45° F. While these inoculations were incubating, effort was made to find out something concerning the coloring of the media. The original culture had retained part of its color at least, through four separate transfers. The known Botrytis culture showed no

coloring, while an isolation from a Delicious apple showed a faint amount of color, and several other spore-streak plates varied in their coloring. The different strains seemed to retain their media-coloring characteristics through numerous transfers in only faintly decreasing intensity.

Daily observations were made on the development of the lesions on the inoculated apples. Spotting appeared along the advancing edge of the rot in all cases during the first few days.

The spotting of the apples kept in the laboratory soon spread out into vague, irregular blotches and became indistinct or disappeared, but could, however, be noted to a slight extent along the advancing edge. There were no differences in any of the inoculated apples kept in the laboratory, either in spotting or in rate of decay. At the end of 22 days the apples in the laboratory were entirely rotted and covered with a grey mass of conidal tufts.

When the apples in the cellar were brought back to the laboratory at the end of 22 days they were one-half to three-fourths rotted. The decayed areas were lighter in color and more translucent than those on the apples held in the laboratory, and were covered with the characteristic spots, even the older spots retaining the sharply defined ring form. As in the laboratory specimens, there were no differences in the various inoculations, all three cultures producing the same kind of spotting.

Re-isolations gave typical *Botrytis* in all cases. The red coloration of the medium was constant, appearing where it had been originally and not appearing where it had been absent. The persistent red color in some cultures of *Botrytis* and its absence in others seems to be characteristic of different cultural strains of the fungus.

SUMMARY

1. The spot-rot of apples is caused by a species of *Botrytis*, probably *B. cinerea*.

2. The Botrytis rot develops best at relatively high temperatures but without the spotting.

3. The spotting of the apple depends on development of the rot at low temperatures. At higher temperatures the spots become indistinct blotches or fade out entirely.

4. The development of the red coloration of the cultures is peculiar to certain strains or isolations.

DEPARTMENT OF PLANT PATHOLOGY,
STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON.

BOOK REVIEW

Fungous Diseases of Fruit-Trees in New Zealand and Their Remedial Treatment. By G. H. Cunningham, B.Sc. Spray formulae in collaboration with the Director, Assistant Director, and Orchard Instructors, Horticulture Division, Department of Agriculture. 382 pages, 177 figures. Published under the authority of the New Zealand Fruit-Growers' Federation, Ltd., by The Brett Printing and Publishing Company, Ltd., Auckland. 1925.

This book was written to meet the needs of fruit growers for information relating to diseases which affect commercial orchards of New Zealand. To this end, the author has sought simplicity of treatment, and a scope and arrangement of material "in accordance with suggestions from the orchardists themselves."

The first or "general" section of the book (99 pages) is devoted to an elementary consideration of the geography and climate of New Zealand, botany of the fruit tree, wounds and their treatment, fungi, bacteria, diseases of plants, and remedial treatments. The second or "special" section (249 pages) deals with specific diseases. These are treated in three divisions: I. pome fruit (apple, medlar, pear, and quince) diseases; II. stone fruit (almond, apricot, cherry, nectarine, peach, and plum) diseases; and III. citrus fruit (grapefruit, citron, lime, lemon, orange, and pomelo) diseases. In each division the diseases are subdivided into three groups, (a) "fungous," (b) "bacterial," and (c) "physiological." At the beginning of each major division are a chart showing the diseases which affect each host and an artificial key for the identification of diseases by symptoms. Each disease is treated in relation to the host group which it most seriously affects. The treatment of each of the more important diseases ordinarily includes consideration of names, hosts, geographic distribution, "appearance and effect on the hosts," life history of the causal organism, and control measures. Following the treatment of each disease are references to citations which are assembled in a bibliography of 337 titles. Technical words are explained in a glossary.

The book is well printed on good paper. The illustrations are numerous and, to an unusual extent, original. Many of the photographs are of excellent quality, although a few are poor. The drawings are semi-diagrammatic. The illustrations are well reproduced. In most cases, however, they would serve their purposes equally well and add to the attractiveness of the work if somewhat reduced in size.

The author is to be congratulated upon the successful way in which he has accomplished a very difficult task. He has combined the simplicity of treatment requisite to the purposes of the book with scientific soundness and accuracy. From the point of view of the fruit growers for whom it was written, the book should be very satisfactory. It should be realized, however, that in a single volume of this length and scope it is impossible to give a full discussion of the practical problems presented by each disease treated. For this more detailed or extended information, it will be necessary to seek special advice or refer to special literature. From the point of view of the plant pathologist, the book presents a welcome and valuable account of the fruit dis-

ease problems of New Zealand and the methods which are being used to meet them. Its limitations are, for the most part, those imposed by its scope and purposes. The treatment of diseases which affect several plants in relation to host groups, rather than individual host plants, for example, precludes giving as much attention as would be desirable to problems which vary with hosts. In the treatment of symptoms and the preparation of keys to identification by symptoms, the author appears to attach more weight to the constancy of symptoms and the fixity of their relations to specific causes than would the present writer. The book shows evidence of much care in preparation and contains few errors in typography or transcription.

The wisdom of the author in seeking the assistance of individuals and organizations in position to contribute to his enterprise and the liberal spirit of cooperation with which they responded merit special note and commendation. This work does credit both to the scientific leadership and the intelligent initiative of the fruit growers of New Zealand.

G. W. KEITT

PHYTOPATHOLOGY

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A BEGONIA IMMUNE TO CROWNGALL: WITH OBSERVATIONS ON OTHER IMMUNE OR SEMI-IMMUNE PLANTS

(The first of a series of Potentiometer Studies.)

ERWIN F. SMITH AND AGNES J. QUIRK

On the crowns of a tall-growing begonia, long cultivated in our houses on account of its large flower clusters and showy foliage, we observed numerous natural galls supposed to be crowngalls. When examined more critically, they were found to be nematode galls due to *Heterodera radicicola*. Subsequently, inoculations of virulent cultures of the hop strain of *Bacterium tumefaciens*, on a large scale, three times repeated, failed to produce any tumors on these plants, although the same cultures inoculated into sunflowers, sugar beets and *Ricinus* developed the usual tumors within the ordinary time (Fig. 2).

This plant, known to us as *Begonia lucerna*, said to be one of the derivatives of *B. coccinea* (*B. rubra* of gardeners), has large, very oblique, smooth leaves which are very red on the under-surface, especially the young leaves, and green above with silver spots (Fig. 1). With plenty of room the plant often grows to a height of 6 or 8 feet or more, and the red flower clusters may be a foot long and equally broad.

The above-mentioned unexpected results led to the following potentiometer tests and other tests. In each case we experimented with 10 cc. of undiluted juice, which was tested in a Hildebrand calomel-cell apparatus as soon as possible after its extraction. The plants were ground in a meat chopper, and the juice was obtained by means of a small hand press. Whenever minutes are used in connection with hydrogen, it means minutes from the beginning of the grinding to the beginning of the hydrogen saturation of the platinized needle. T. = Temperature in degrees Centigrade; mv. = millivolts; and T.a. = Total acidity in terms of Fuller's scale, *i.e.*, number of cubic centimeters of $\frac{N}{1}$ NaOH required per liter of juice to bring the fluid from the given pH to pH 8.2 (phenolphthalein neutrality). In each

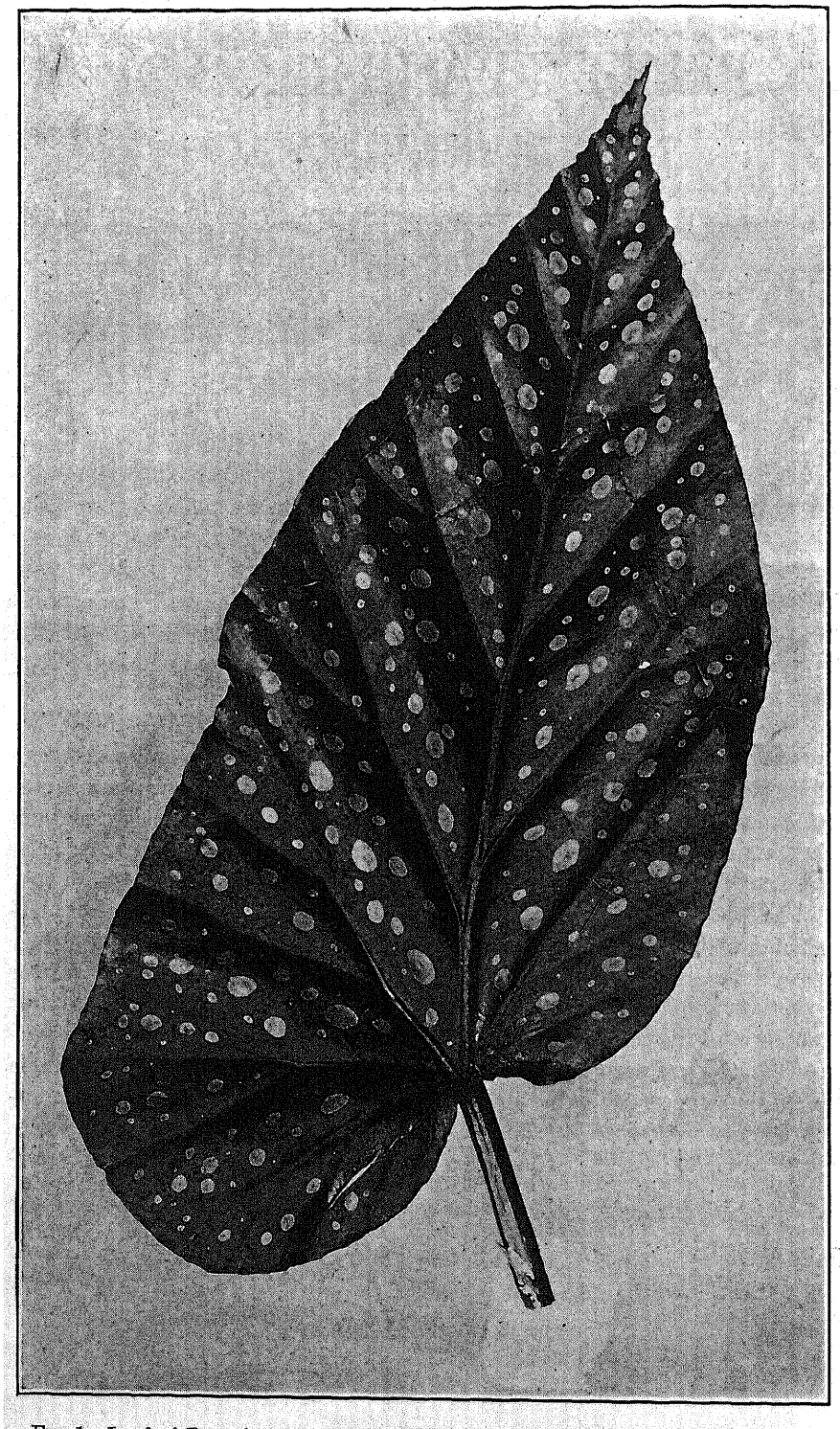

FIG. 1. Leaf of *Begonia lucerna*, 7/8 natural size, showing the numerous silver spots.

case the readings on the potentiometer were continued until there was no further rise in potential, and the highest reading was always taken for computing the pH by means of the constants of Michaelis.

Exp. 157. March 20, 1924, 10:30 A. M. Removed and ground 50 large upper leaves from numerous shoots. None flabby or yellowed. Weight 175 grams. With moderate pressure we obtained about 100 cc. of a strongly acid, non-viscid, opaque fluid, the color of capillary blood. Hydrogen on in 18 min. T. 26; mv. 324.2; pH 1.35; T.a. + 247 (estimated; too much alkali was added at the end). This result, only approximately true with respect to total acidity, was so astonishing that the experiment was immediately repeated, with the following result.

Exp. 158. The acid fluid was centrifuged and we obtained a clear blood red fluid which tasted a little like currant juice, *i.e.,* it was strongly acid and somewhat acerb. Hydrogen on in 115 min. T. 26; mv. 325.5; pH 1.36; T.a. + 263.

Exp. 159. The soft green tops of 40 shoots of *Begonia lucerna* were ground after removal of the leaves. Weight 333 grams. From these under moderate pressure we obtained 200 cc. of a cloudy, dirty, yellowish green, non-viscid fluid containing only a trace of pink color. After centrifuging, the fluid was still cloudy and of a pinkish yellow color. Hydrogen on in 25 min. T. 25; mv. 377.8; pH 2.23; T.a. + 99.

Exp. 160. Bases of 76 shoots taken from about a dozen pots. Nine-tenths of these shoots were covered with a smooth brown-purple bark, the rest were green on the surface, a few were rather woody. All of these plants had nematode tumors at the surface of the earth and below. Weight 390 grams. Extracted 210 cc. of a yellowish green fluid, which was still cloudy and yellowish green after centrifuging. Hydrogen on in 32 min. T. 25; mv. 447.8; pH 3.42; T.a. + 40.5.

Exp. 161. Nematode tumors from the bases of 36 stems. All small, considerable necrosis in each one. Many others were too badly decayed to use. We obtained only a small double handful of usable galls, few of which were perfectly sound. Extracted about 60 cc. of a dirty, yellowish-white, very cloudy fluid from which a muddy brown precipitate settled out at once. On centrifuging there was an additional dirty precipitate containing a little starch, but the fluid remained thinly clouded and whitish. Hydrogen on in 18 min. T. 26; mv. 458.4; pH 3.60; T.a. + 32.

Somewhere between pH 4.28 and pH 4.80 in the titration curve (sodium hydroxide being added) the color of this tumor juice changed from white to blue, but at the end of the titration (pH 8.2) it was dark red. This result appeared so inexplicable that the experiment was repeated to see if the hastily recorded first dark color (blue) was not an erroneous determination.

Exp. 162. Into 10 cc. of the white fluid of Exp. 161 we allowed $\frac{N}{20}$ NaOH

to stream slowly. At first the thin stream of clear alkali looked like a smoke cloud as it entered the juice. When enough had been added, the

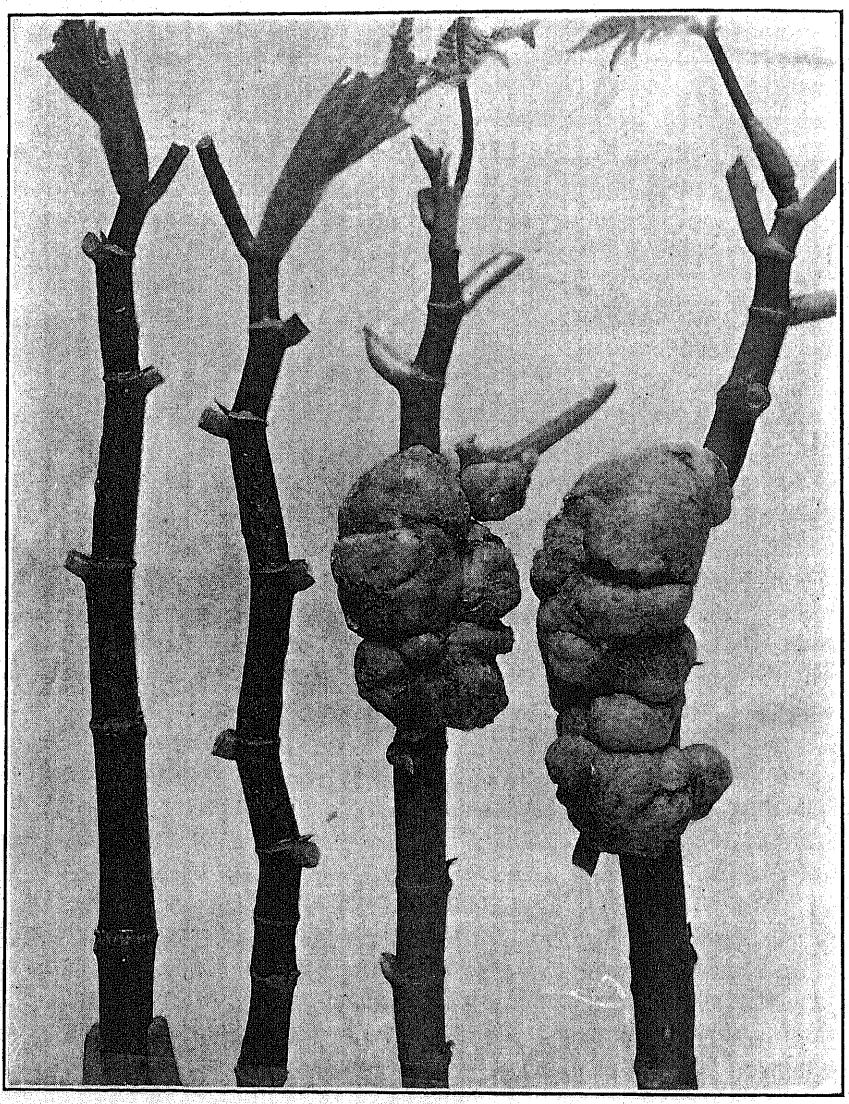

FIG. 2. Left: Shoots of *Begonia lucerna* (fourth series) inoculated copiously (20 needle pricks) with *Bact. tumefaciens*, hop strain. Right: two controls on *Ricinus* (inoculated from the same tube). Time six weeks (nearly). The whitish patches on the begonia shoots are masses of bacteria through which the punctures were made. 2/3 nat. size.

fluid became a pale blue; with further additions of alkali the fluid became first a deep blue, then purple, and finally a deep red.

Exp. 163. We now took the juice of Exp. 160 (base of the shoots) and allowed the dilute alkali to stream into it in the same way. After a time it also became pale blue, then deep blue and finally red as in Exp. 161 and 162.

Exp. 164. The test was now repeated on juice from the top of the shoots (Exp. 159), with the same result.

Exp. 165. A final test was made on 10 cc. of the centrifuged blood red juice from the leaves (Exp. 158). On addition of $\frac{N}{1}$ NaOH the clear, blood red fluid apparently behaved differently (see later), the first observed change being a purple-color, then brown, then darker brown, then dirty bluish, and finally greenish. We continued to add alkali but with no production of any final red color. It stayed in the green.

The following year, 1925, the juice of leaves and stems of this plant was extracted, centrifuged, titrated electrometrically with sodium hydroxide down to a pH within the limits of growth of *Bact. tumefaciens* (its bouillon range as determined in this laboratory is approximately pH 5.70 to pH 9.20), sterilized, retested in order to determine that the titre had not changed, and inoculated with the hop strain of *Bact. tumefaciens.* In the juice thus treated the crowngall organism grew readily. One of these juices registered pH 6.56, the other pH 7.10, and the best growth was in the latter. In the third set, to which an excess of alkali had been added, there was a very copious precipitate on steaming, leaving a colorless fluid (the others were dark) the final titre of which was decidedly more acid (pH 4.55) than before the steaming; and in this fluid, as we expected, the organism would not grow. Before adding alkali the acidity of these juices as determined on the potentiometer was as follows:

Exp. 521. Blood red juice from the upper leaves, pH 1.06.

Exp. 522. Pale juice from soft tops of shoots, pH 1.33.

On adding clear lime water to the acid juice a very copious precipitate of insoluble calcium oxalate was obtained, *i.e.,* the washed white precipitate was insoluble in oxalic acid or acetic acid, but readily soluble in dilute hydrochloric acid. A part of the acid, therefore, is oxalic acid, but not all of it, since lime salts remained in solution in the fluid, as shown by evaporating some of it.

Some weeks later, August 18, 1925, a third set of tests was made on the leaf, stem, and nematode tumor juices of *Begonia lucerna.* At 11:15 A. M. we cut 21 shoots, which were brought into the laboratory and tested with the following results:

Exp. 537. From the 21 shoots we removed 64 upper leaves and commenced to grind them at 11:30 A. M. Extracted 190 cc. of red, acid juice.

Centrifuged 10 minutes. Fluid a clear blood red. Hydrogen on in 65 min. Fluid not viscid. T. 29; mv. 323.5; pH 1.34; T.a. (lost)—too much alkali was added at the end, *i.e.*, 0.5 cc. of $\frac{N}{1}$ NaOH carried the record from mv. 640.5 (pH 6.64) to mv. 912.5 (= pH 11, color then brownish green), the desired reading for pH 8.2 being mv. 733.78. T.a. beyond + 250.

Exp. 538. Duplicate of Exp. 537. Hydrogen on in 90 min. T. 29; mv. 313.0; pH 1.31; T.a. + 264.

Some preliminary tests were made on the juice from these leaves by slowly adding $\frac{N}{20}$ NaOH. The first color (on the border line of the two fluids) was a fugitive blue, overlooked when the stronger alkali was used in 1924 (Exp. 165), then purple, then brown, becoming, with more and stronger alkali $\left(\frac{N}{1}\right)$, yellowish green.

Exp. 539. Commenced to grind the 21 soft green shoots at 2 P. M. Extracted 245 cc. of a turbid yellow fluid. Centrifuged 10 min. Fluid a clear salmon color (yellowish pink). Hydrogen on in 48 min. T. 30; mv. 349.8; pH 1.79; T.a. + 118. At mv. 505.7 (pH. 4.39) there was a slight change in color which deepened at mv. 518 and became definitely blue at mv. 554 (pH 5.19). At mv. 622 (pH. 6.32) the fluid was a deep violet blue. At mv. 663.6, reddish blue (Quirk), purple red (E. F. S.). At mv. 911 (pH 11) this fluid was brown red, whereas the juice from the leaves was brown green (see Exp. 537).

Exp. 540. Tested a double handful of nematode galls collected in fresher condition than those of 1924. Commenced to grind at 4 P. M. Obtained 110 cc. of a yellow milky juice. Centrifuged 10 minutes. A copious dirty precipitate and a translucent milky yellowish fluid. Hydrogen on in 30 min. Fluid not viscid. T. 30; mv. 426.8; pH 3.07; T.a. + 54. At mv. 523 (pH 4.68) the first distinct pale blue color; at mv. 563.5 (pH 5.36) the fluid became deep blue; at mv. 608.5 it was purplish; at mv. 631, reddish; from mv. 649 on, the fluid was red.

The following additional tests were made on August 31, 1925.

Exp. 544. Soft tops of 24 shoots (leaves removed). Cut at 11:50 A. M. Commenced to grind at 12:10 P. M. With moderate pressure we obtained 182 cc. of an opaque, olivaceous juice. Centrifuged 10 min., fluid a clear pink. Hydrogen on in 27 min. T. 29; mv. 350.2; pH 1.79; T.a. + 135 (estimated; too much alkali was added at the end). No change in color from mv. 350 (pH 1.79) to mv. 465 (pH 3.71); at mv. 476 (pH 3.90) a trifling change toward blue; at mv. 506 (pH 4.40) still pink; at mv. 532 (pH 4.83) the neutral point not yet reached; between this pH and mv. 613.8 (pH. 6.19) the preceding color changed to blue; at the end point (pH 8.2) it was red.

FIG. 3. Inoculated slow-growing crowngalls on the B. P. I. hybrid begonia and leaf of the same. Juice pH 1.65. Time 4½ months. Photo. Jan. 20, 1926, 7/8 natural size.

Exp. 545. Duplicate of Exp. 544. Hydrogen on in 65 min. T. 30; mv. 350.2; pH 1.79; T.a. + 140. At mv. 584 (pH 5.69) pale blue; at mv. 606.6 (pH 6.07) deep blue; at mv. 635.7 (pH 6.55) violet blue; at mv. 670.7 (pH 7.14) purple blue; at mv. 686.5 (pH 7.40) purple red; between mv. 722.6 and mv. 742.0 (pH 8.00 to 8.32) red.

Exp. 546. Bases of 24 old stems (9 only with brown bark). Commenced to grind at 2:00 P. M. Extracted 225 cc. of opaque sulfur-green juice. Centrifuged 10 min. Fluid still opaque and yellow. Hydrogen on in 49 min. T. 31; mv. 441.5; pH 3.30; T.a. + 56. At mv. 540 (pH 4.84 the fluid changed to blue, at mv. 586 (pH 5.72) it was a deeper blue; at mv. 636.7 (pH 6.57) it was violet blue; at mv. 698 (pH 7.58) it was red purple; and at mv. 739.7 (pH 8.28) it was red.

Exp. 547. Duplicate of Exp. 546. Hydrogen on in 80 min. T. 31; mv. 440; pH 3.30; T.a. + 56. Color bluish at mv. 532 (pH 4.83); deep blue at mv. 574.7 (pH 5.54); deep blue at mv. 653 (pH 6.84); red at mv. 714 (pH 7.85) and onward to mv. 731 (pH 8.11).

TABLE 1.—*The H-ion concentration and total acidity of the juice of Begonia lucerna*

Experiment No.	Degrees Centigrade	Millivolts	pH	Total acidity (undissociated salts)
Leaves				
157	26	324.2	1.35	+ 247 estimated
158	26	325.5	1.36	+ 263
521	.....	.....	1.06	.....
537	29	323.5	1.34	Lost (250 plus)
538	29	313.0	1.31	+ 264
548	30	296.0	0.90	+ 250
Top of shoots				
159	25	377.8	2.23	+ 99
522	.....	.....	1.33	.....
538	30	349.8	1.79	+ 118
544	29	350.2	1.79	+ 135 (estimated)
545	30	350.2	1.79	+ 140
Base of shoots				
160	25	447.8	3.42	+ 40.5
546	31	441.5	3.30	+ 56
547	31	440.0	3.30	+ 56
Nematode tumors on crowns				
161	26	458.4	3.60	+ 32 (somewhat decayed)
540	30	426.8	3.07	+ 54
600	24	458.5	3.60	+ 35
601	24	458.6	3.67	+ 35

TABLE 2.—*Behavior of Bact. tumefaciens (hop strain) in a graded series of peptone beef bouillons, inoc. Aug. 28, 1925. First examination at end of 24 hours at 25° C.*

pH (colorimetric)	Behavior	pH of controls on Sept. 2 (potentiometer)
5.2	Aug. 29 Both clear do 31 do Sept. 2 do ck. clear	.....
5.4	Aug. 29 Both clear do 31 do Sept. 2 do ck. do	.....
5.5	Aug. 29 Both clear do 31 do Sept. 2 do ck. do	.....
5.6	Aug. 29 Both clear do 31 do Sept. 2 do ck. do (uncorrected bouillon)	5.6
5.8	Aug. 29 One cloudy, one clear. The cloudy tube received the most inoculum do 31 One cloudy, one clear Sept. 2 Both cloudy with a good pellicle, ck. clear	5.7
6.1	Aug. 29 Both cloudy do 31 Cloudier with pellicle Sept. 2 Both well clouded with good pellicle, ck. clear	.....
6.3	Aug. 29 Both cloudy with flocculence do 31 Better growth. Pellicle Sept. 2 Both well clouded. Good pellicle, ck. clear	.....
7.0	Aug. 29 Best growth. Well clouded, thin pellicle do 31 do do. good do Sept. 2 Heavy pellicle in each tube. Fluid beginning to clear, ck. clear	.....
8.6	Aug. 29 Inoc. to-day from pH 7.0 of Aug. 28 do 31 Both cloudy Sept. 2 Each well clouded with pellicle. Less clouded now than in pH 6.3, ck. clear	.....
9.0	Aug. 29 Both cloudy with plain rim breaking up on shaking do 31 Heavier growth with pellicle Sept. 2 Moderate clouding, good pellicle, ck. clear	.....
9.4	Aug. 29 One tube thinly clouded, one clear do 31 Both clouded with pellicle Sept. 2 Good pellicle, twice as much clouding as in pH 9.8, ck. clear	8.84
9.8	Aug. 29 Both clear do 31 do Sept. 2 Both clouded with a thin pellicle, ck. clear	9.05

In March, 1926, another test was made of juice from nematode galls on this begonia with the following results:

Exp. 600. Hardly any starch in the precipitate, *i.e.*, not more than 1/30 of the whole. Fluid slightly cloudy and pale yellowish. Hydrogen on in 33 min. T. 24; mv. 458.5; pH 3.60; T.a. + 35.

Exp. 601. Duplicate. Hydrogen on in 59 min. T. 24; mv. 458.6; pH 3.67; T.a. + 35. At pH 4.50 the fluid was still yellow; at pH 5.48 it was blue; at pH 7.00 it was violet red; and at pH 7.53 and on, it was red.

For the convenience of the reader the potentiometer determinations are assembled in table 1.

Up to this time we had data on the behavior of *Bact. tumefaciens* in acid media only, as given by Quirk and Fawcett.[1] Those data leave a gap between pH 5.7, at which the organism grew, and pH 5.2, at which no growth was obtained. To fill this gap and also to redetermine its behavior in alkaline bouillons we prepared a series of graded peptone beef infusion bouillons and inoculated them immediately after the pH tests were made. The inoculum was, in each case, a clearly visible small quantity of bacterial growth from an 8-day agar streak (all from the same tube) carried on the tip of a platinum needle. Two tubes of each grade were inoculated and a third was held as a control. The results, confirmatory of the earlier observations, are given in table 2.

Some change toward the acid side allowing growth might have occurred over night in pH 9.4, before the clouding was observed on Aug. 29, as the strongly alkaline beef bouillons do not remain very long where they are put.

On Sept. 2 at 4 P. M. four additional alkaline bouillons were tested on the potentiometer and immediately inoculated. Temp. 25° C. Two cks. held

TABLE 3.—*The reaction of Bact. tumefaciens in alkaline peptone bouillons*

pH	Behavior	
9.00	Sept. 3	10 A. M. One thinly clouded, one clear (that receiving the the least inoculum).
		4 P. M. Both thinly clouded, no rim or pellicle.
	do 4 and 5	Both thinly clouded, rolling clouds on shaking, trace of rim.
	do 8	Good pellicle on each one. Checks clear.
9.19	Sept. 3 and 4	Both clear.
	do 5	3 P. M. Both thinly clouded.
	do 8	Cloudier than on Sept. 5, but thin. Both checks clear.
9.41	Sept. 3 to 9	Both clear. Checks clear.
9.65	Sept. 3 to 9	Both clear. Checks clear.

[1] Quirk and Fawcett. Hydrogen-ion concentration vs. titratable acidity in culture mediums. Jour. Inf. Diseases 33: 48. 1923.

on each pH and two tubes inoculated, one much heavier than the other. Each received a 3 mm. loop of inoculum from bouillon pH 6.3 (preceding series), and one also a good sized fragment of pellicle, *i.e.*, 50 to 100 times as many bacteria as the other. The results are given in table 3.

EXPERIMENTS ON OTHER BEGONIAS

The foregoing experiments led to tests of several other begonias which likewise were found to be very acid but not entirely resistant to crowngall inoculations.

The Phyllomaniac Begonia

Exp. 532. Aug. 10, 1925, 11:10 A. M. Brought in 46 young shoots of *Begonia phyllomaniaca* and removed the leaves. Weight of the shoots 512 grams. Commenced to grind at 11:40 A. M. Extracted under moderate pressure 390 cc. of a muddy green fluid. The first taste was strongly acid followed by a bitter taste like quinine or quassia. Hydrogen on in 45 min. T. 30; mv. 358.5; pH 1.94; T.a. lost (too much alkali added at the end). The proper record was somewhere just beyond + 150.

Exp. 533. Duplicate of Exp. 532. Hydrogen on in 127 min. T. 30; mv. 356.9; pH 1.91; T.a. + 153. At mv. 506 (pH 4.35) the pink color disappeared, and at mv. 596 (pH 5.83) the fluid became blue.

Exp. 534. Juice of about 300 leaves from the 46 shoots. They had been kept closely wrapped and were not wilted. Commenced to grind at 2 P. M. Extracted 470 cc. of a muddy chocolate brown fluid. Centrifuged 10 minutes. The fluid was now cinnamon color and still opaque. It was centrifuged another 10 min. (3,000 rev. per min.) without change of color or opacity. Hydrogen on in 69 min. T. 31; mv. 333.0; pH 1.52; T.a. + 206.

On adding 250 cc. of lime water to an equal volume of the stem juice, we obtained evidence of oxalic acid, *i.e.*, an immediate copious white precipitate soluble in dilute HCl but insoluble in oxalic acid or glacial acetic acid. The leaf juice treated in the same way became milky cloudy but did not precipitate for a long time, not even on adding another 150 cc. of the lime water and finally 240 cc. more. On centrifuging, the fluid partly cleared, yielding a dirty white precipitate, part of which dissolved in HCl.

We had no expectation of obtaining crowngalls on these plants, but inoculations from agar streaks on September 3, 1925, made in the soft upper stems of about 20 of these plants with the hop strain of *Bact. tumefaciens*, produced slow-growing tumors on most of them. Those that failed to produce tumors were plants that grew poorly, and rotted off at the base a little later from overwatering and the attack of *Fusarium*. These tumors were small and thickly studded with shoots (Fig. 4B).[2]

[2] For numerous photographs and observations on this strange plant see Bacterial Diseases of Plants, pp. 574–621. W. B. Saunders Co., Phila. and London, 1920.

The Vernon Begonia

The following experiments were made with the Vernon begonia, a favorite bedding plant on account of the profusion of rose red to rose pink flowers (R_2). The leaf blades, seldom more than 2 to 2½ inches broad and not much longer, are fine, doubly serrate and very oblique (Fig. 5). They are a glossy metallic green above and a dull green below with traces of red, petioles pink. The stems are greenish or reddish. Through a hand lens the under surface of the leaf blade shows innumerable small roundish white spots, which, under the compound microscope, are seen to be islands of stomata. The plant is smooth except for scattered hairs.

Exp. 544. Ground 20 fresh shoots of the Vernon begonia (leaves removed). Extracted 130 cc. of an opaque cinnamon red juice. Centrifuged 10 min. Fluid now a clear bright red. Owing to an accident the juice was set into the icebox over night, and the following tests were made the next morning.

Exp. 548. T. 29; mv. 340.8; pH 1.63; T.a. ————.

Exp. 549. Duplicate of Exp. 548. T. 29; mv. 340.8; pH 1.63; T.a. + 191.

Two other tests of the total acidity gave + 193 and + 194.

Here again, from the results on *Begonia lucerna,* we had no expectation of getting any tumors from needle pricks introducing *Bact. tumefaciens* (hop strain), but after some months a few small tumors developed (Fig. 4C).

B. P. I. Hybrid Begonia

On September 3, 1925, crowngall inoculations were also made on the soft upper part of four plants from a hybrid begonia of uncertain parentage. It was produced in the Bureau of Plant Industry, but we have not learned who made the cross. Only these four plants were available for the experiment. No positive results were expected, but slow-growing tumors developed on each one of the four plants (Fig. 3). The plants continued to grow beyond the point of inoculations, and potentiometer tests of the tops of these plants made on January 20, 1926, gave the following results.

(1) Ground upper stem of the 4 plants, *i.e.*, above the tumors (leaves removed), and extracted 55 cc. of opaque cinnamon-colored juice. On centrifuging, a little starch and chlorophyll were thrown down and a clear scarlet red acid fluid remained with the following reactions: T. 25; mv. 341.8; pH 1.62; T.a. + 156, approximate. Duplicate, T. 25; mv. 342.6; pH 1.60; T.a. + 154. Second dupl., T. 25; mv. 343.6; pH 1.65; T.a. + 153.

(2) Forty-eight leaves of the same. T. 25; mv. 342.8; pH 1.66; T.a. + 160.

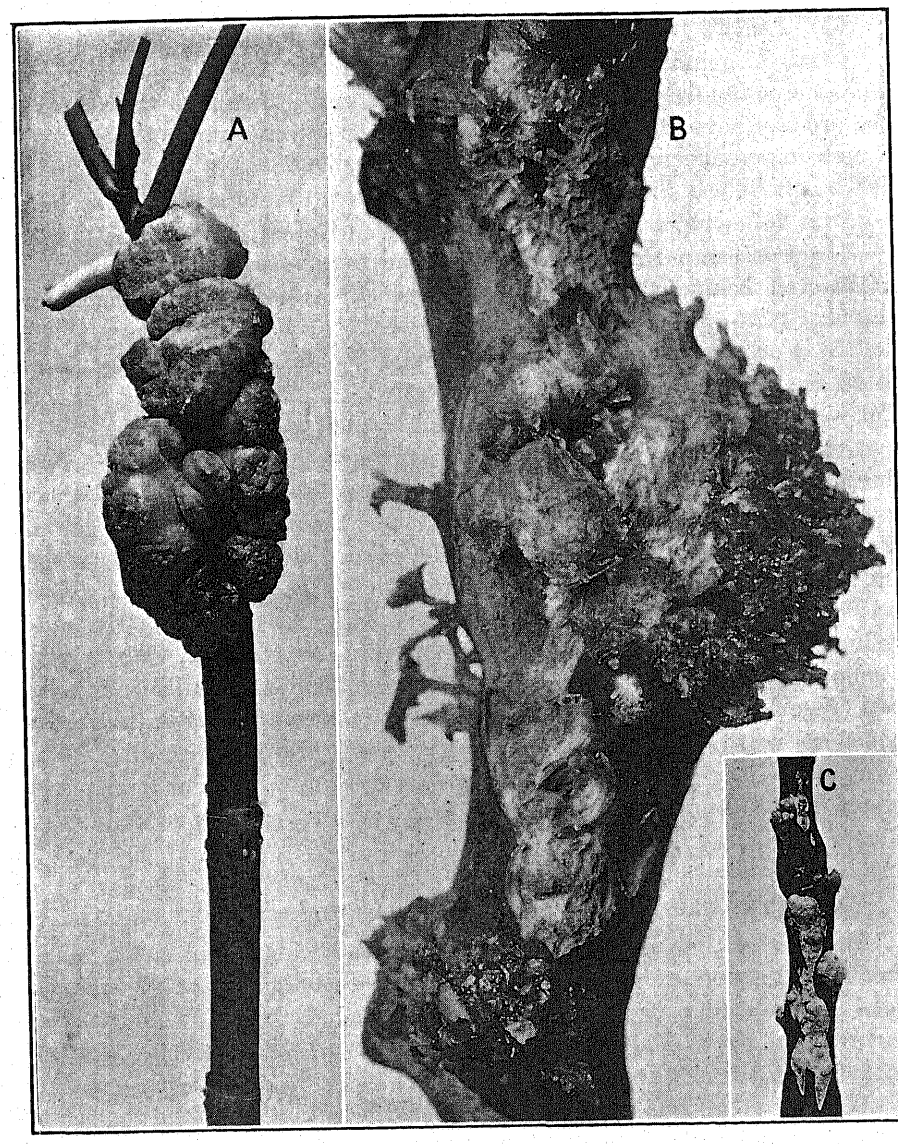

FIG. 4. A. Tumor on *Ricinus* (2/3 natural size) at end of 30 days for comparison with tumors shown in figure 3 at end of 4½ months. This tumor was produced with a sub-culture of col. 4 obtained from the tumors shown at the right in figure 3.

B. Inoculated slow-growing crowngall on *Begonia phyllomaniaca*. Right side of tumor full of tiny shoots. Juice pH 1.94. Time 84 days. Photo. Nov. 27, 1925. Twice natural size.

C. Slow-growing crowngalls on Vernon begonia. Juice pH 1.63. Time 100 days. 2/3 natural size. It may be compared with A.

(3) Tumors from the same. Only 2 cc. of a cloudy milky juice was obtained on grinding and extracting, and part of this came from normal tissues surrounding the tumors, and this naturally increased the acidity of the product beyond that which the pure tumor juice would have given. As it was impossible to test so small a quantity as 2 cc. in our calomel cell apparatus, it was diluted with distilled water up to 10 cc. and then tested with the following results: T. 25; mv. 512.0; pH 4.50; T.a. +45.

(4) For comparison with No. 3. The four stems immediately above, below and behind the tumors were crushed, extracted, centrifuged and tested. T. 25; mv. 396.0; pH 2.54; T.a. + 88.

(5) For an exact comparison with No. 3, 2 cc. of No. 4 were now diluted to 10 cc. with the distilled water and tested. T. 25; mv. 420.9; pH 2.96; T.a. + 80, appr. Dupl. T. 25; mv. 420; pH 2.94; T.a. + 84, appr.

It is thus evident that the normal acidity of the begonia tissue was reduced locally by the growth of the tumor and this is in keeping with our experiments on other plants. For example the juice from soft Ricinus stems like those shown in figures 2 and 4 has a pH of 5.53 to 5.54 (several tests), whereas the tumor juice is more alkaline, ranging from pH 5.74 to 5.79 (several tests). To what extent the acidity of the begonia tissue was reduced it is impossible to determine from this experiment. We think to somewhere between pH 5 and pH 6. Cultures from the smaller (left hand) gall (Fig. 3) yielded nothing on the agar plates, nor anything in the original bouillon, in which the whole of it was crushed. Mixed juice of the other 3 tumors (a loop full) into bouillon yielded typical colonies on agar poured plates with subcultures, from one of which the tumor was reproduced on *Ricinus* (Fig. 4A).

ACIDITY OF OTHER PLANTS IMMUNE OR SEMI-IMMUNE TO CROWNGALL

For a long time we have known that olive shoots, onion shoots, garlic shoots, and shoots of various other plants, especially monocotyledons, can not be inoculated very successfully with crowngall (many unpublished experiments of Erwin F. Smith and Nellie A. Brown), but this is the first time potentiometer tests have been made.

Without going into details it may be said here that we found the following conditions in the juice of the plants known or supposed to be resistant to crowngall:

Pink skinned, small, very juicy onions (Allium cepa): pH 5.15. Total acidity, lost (a little too much alkali was added and the final reading was pH 8.54 (T.a. + 50)). Dupl. pH 5.02. T.a. + 43.

Green tops of brown skin onions: pH 4.33; T.a. + 30. Dupl. pH 4.38; T.a. + 32.

Green tops of silver skin onions: pH 4.45; T.a. +30. Dupl. pH 4.50; T.a. +34.

White dry garlic bulbs (Allium sativum) from Italy: pH 3.53. T.a. lost. Dupl. pH 3.46; T.a. +165.

Elephant's-ear (Colocasia antiquorum) leaves: pH 5.33; T.a. + 10.

Eleven petioles from 6 small plants, pH 5.72; T.a. +9. Dupl. pH 5.71; T.a. +8.

Three inner petioles from 2 large plants, pH 5.65; T.a. +8. Dupl. pH 5.61; T.a. +8.

Young shoots of olive (Olea europea): pH 5.22; T.a. +52; Dupl. pH 5.20; T.a. +52; 2nd dupl. pH 5.28; T.a. +52.

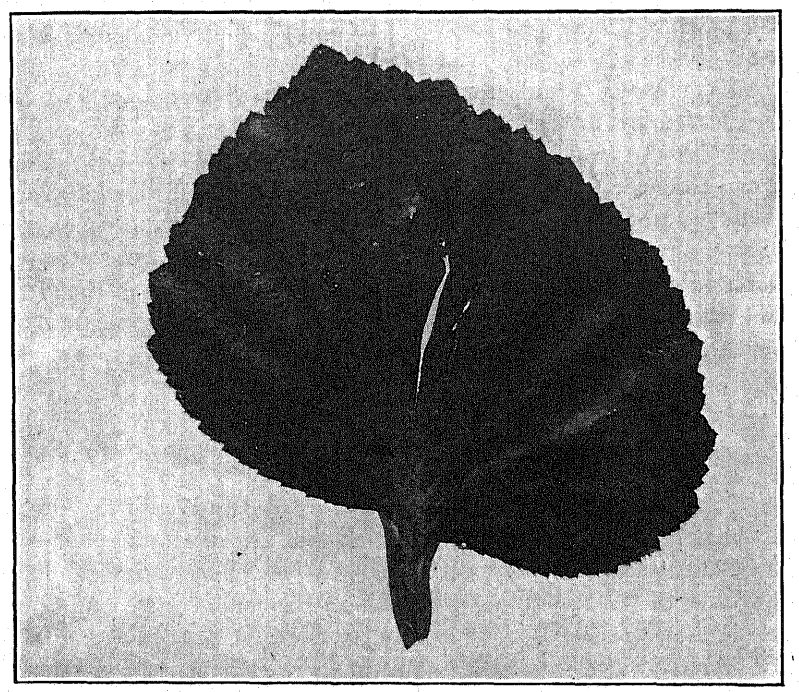

FIG. 5. Leaf of Vernon begonia (natural size). For stem see figure 4C.

Young petioles and midribs of banana (red variety): pH 5.02; T.a. +14. Dupl. pH 5.04; T.a. +14.

Young petioles and midribs of yellow fruited banana (Gros Michel): pH 4.74; T.a. +12. Dupl. pH 4.70; T.a. +12.

Juice of soft tops of sugar cane (var. Lahina), leaves excluded: pH 4.88; T.a. + 37. Dupl. pH 4.91; T.a. + 37. Another set: pH 4.82; T.a. + 60; dupl. pH 4.82; T.a. + 63.

The Wandering Jew (Tradescantia zebrina or Zebrina pendula) leaves: These leaves were broadly lanceolate, white-striped above and purple underneath. pH 5.47; T.a. between +8 and +10. Dupl. pH 5.44; T.a. +8.

Stems: pH 5.52; T.a. +21. Dupl. pH 5.52; T.a. +21.

Juice of young pineapple leaves (the smooth leaved variety commonly cultivated in the Panama Canal Zone): pH 4.72; T.a. between +38 and +40. Dupl. pH 4.73; T.a. +39.

Boston Market fern (Nephrolepis exaltata var. Bostoniensis): (32 fronds) pH 5.20; T.a. +38. Dupl. pH 5.22; T.a. +38.

Holly fern (Polystichum lonchitis, also called Aspidium): (25 fronds) pH 5.35; T.a. between +41 and +42. Dupl. pH 5.32; T.a. +42.

Rumex crispus (leaves): pH 3.53; T.a. +66. Dupl. pH 3.56; T.a. +66.

Rumex obtusifolius (40 leaves) pH 3.64; T.a. +68. Dupl. pH 3.67; T.a. +69.

Hothouse Oxalis (acaulescent, big-leaved, pink-flowered form). Several hundred leaves, pH 1.70; T.a. +224. Dupl. pH 1.72; T.a. +224.

Agave (young leaves): pH 4.30; T.a. +100. Dupl. pH 4.31; T.a. +102.

At the time the tests were made the H-ion ranges of all of these plants were believed to be too acid for the growth of the crowngall organism, and probably the tumor never does occur on them in nature, but we now think it possible to produce it on many of them by excessive doses of the organism. It is not unlikely that the preponderant kind of acid they contain has more to do with the resistance than the total acidity.

SUMMARY OF RESULTS

Begonia lucerna

(1) This is the most acid plant we have ever tested and the concentration of the acid is surprising; (2) the leaves are the most acid part; (3) the acidity of the stem decreases from top to base, *i.e.*, from young to old parts; (4) the juice of the leaves was twice as acid as lemon juice and almost or quite as acid as N/10 HCl;[3] (5) a very considerable part of the acid of this begonia is oxalic acid, but other acids are present; (6) the old nematode tumors were the least acid parts of the plant both in pH value and by electrometric titration; (7) inasmuch as pH 5.70 represents about the limit of growth of *Bact. tumefaciens* in bouillon media and in acid juices, the reason for its failure to grow in this begonia was assumed to be the acidity

[3] The juice of ripe lemons was found to be pH 2.20 (one test and two duplicates, in which we got precisely the same readings). The most astonishing discovery was the buffer content of the lemon juice, the total acidity, as determined by titration on the potentiometer down to pH 8.2 (phenolphthalein neutrality), being around +950 on Fuller's Scale, *i.e.*, it would take 950 c.c. of N/1 NaOH added to 1 liter of the juice to reach the pH 8.2 point in the titration curve.

of its tissues, since it grows readily in the juice of *Begonia lucerna* when its acidity is reduced to pH 6.56, and still better when it is reduced to pH 7.10; (8) *Heterodera radicicola* tolerates a more acid substratum than *Bact. tumefaciens,* and like the latter, when it is able to grow, changes the substratum to suit its needs, *i.e.,* makes it more alkaline when it is too acid; (9) exposure of the expressed juice of this begonia to the air for an hour or two made no appreciable change in its acidity (H-ion content); (10) there are two interesting indicators in this plant. The one in the stem juice is colorless between mv. 349 and mv. 461 (pH 1.77 to pH 3.71); pale blue becoming pure blue between mv. 505 and mv. 554 (pH 4.39 to pH 5.75); deep violet blue at mv. 622 (pH 6.32); and purple red changing to brown red between mv. 663 and mv. 911 (pH 7.01 to pH 11). The end term of the other indicator is brown green.

Other Begonias

What proved true of *B. lucerna* was found to be true of all the other begonias tested; they all have a strongly acid juice. All contain oxalic acid but the relative amounts are not known. All are rather resistant to crowngall inoculations, but this resistance may be overcome by mass action of the bacteria to some extent at least.

Other Plants

All the other plants known or believed to be immune to crowngall were found to have a juice considerably more acid than pH 5.70, the limit of crowngall toleration in bouillon, but all, with the exception of the *Oxalis,* were less acid than the begonias. The plants tested were *Oxalis,* onion, garlic, olive, pineapple, banana, sugar cane, elephant's-ear, *Rumex* (2 sp.), agave, and two ferns.

Assumptions as to pH

We started out with the assumption that the pH controlled tumor formation and that no tumors could be produced on plants having a greater acidity than pH 5.00, but the final experiments show that we have been able to produce crowngall tumors on three begonias having a very much more acid juice reaction than any the organism will tolerate in fluid cultures. The only conclusion we have come to is that mass action must control infection, *i.e.,* if you can once get a tumor started in a tissue, even in a very acid tissue, it will make its own milieu and continue to grow in spite of the inhibiting pH. This probably would be true even on *Begonia lucerna.*

Considering these results, it is premature to state that no crowngall tumors can be produced on the plants mentioned and supposed hitherto to be immune owing to their acid juice. Further experiments should be made, but it is still believed that crowngall tumors on such plants are non-existent

in a state of nature. Other factors than acidity of course may determine resistance in some of these plants.

LABORATORY OF PLANT PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

FUSARIUM ROT OF GLADIOLUS CORMS

L. M. MASSEY[1]

The two most important fungous diseases of gladiolus corms are those to which the names hard rot (2) and dry rot (3) have been given. These are to be found in practically all collections of any size, whereas a third disease, Fusarium rot (4), is sometimes present in sufficient amount to be given equal rank in importance.

Fusarium rot was observed as early as 1912. Although it received casual attention over a period of several years, no special investigation of it was undertaken by the writer until 1916. At about this time what seemed to be the same fungus was isolated from so many diseased corms sent to the Department of Plant Pathology at the New York State College of Agriculture that it was considered advisable to make a more intensive study of the disease and its cause.

Diseased corms have been received from a sufficiently large number of growers in New York to warrant the conclusion that Fusarium rot is of general occurrence in this state. Corms affected with the disease also have been received from Maryland, District of Columbia, New Jersey, Ohio, Indiana, Michigan, Wisconsin, Minnesota, California, and Ontario, Canada. Whether or not the plants were grown by the growers who sent the diseased material has not always been clear. However, with the general practice of growers buying relatively large quantities of corms from one another, it seems probable that diseased corms have been distributed throughout the United States and that the disease occurs in some degree in all areas suitable to the growth and parasitism of the fungus.

No instances of a high percentage of diseased corms in large collections, as is common in the case of both the hard rot and the dry rot, have come to the attention of the writer. In these latter diseases, especially hard rot, it is not uncommon to find over 90 per cent diseased corms in collections numbering several hundred thousands or more. However, three cases have been noted in which over 75 per cent of certain small lots of corms have been found to be affected with Fusarium rot. It is interesting to note that, whereas inoculation experiments indicate the absence of varietal differences

[1] The writer is indebted to the Boyce Thompson Institute for Plant Research for laboratory and greenhouse facilities placed at his disposal during part of the course of this investigation.

in susceptibility, yet in these three cases the disease was more or less restricted to certain varieties.

Although certain workers have given attention to gladiolus diseases, literature contains no reference to this particular disease other than that made by the writer in 1922 (4). It is probable, because of the relatively greater importance of hard rot, dry rot and neck rot or scab (1), that these diseases have received first attention and that one or more of them were involved in cases where the identity of the disease is left uncertain in published accounts.

SYMPTOMS OF THE DISEASE

At the time of harvesting the corms in the autumn, Fusarium rot lesions appear as small water-soaked spots. They are of a reddish-brown color and occur in most cases on the sides and lower halves of the corms (Pl. XXV). It is usually necessary to remove the husks (sheathing leaf bases) from the corms in order to see the lesions, although in some cases the husk is also diseased, as indicated by its discoloration and unusual brittleness.

The rot advances during storage, the rate increasing with higher temperatures and increased humidities. Lesions are usually irregularly circular in outline and range in size from one-fourth to one inch in diameter depending upon conditions of storage and time of year. The entire corm may become involved and reduced to a dry, brownish-black, worthless mummy due to the coalescence of two or more lesions.

Perhaps the most characteristic symptom of this disease is the existence of prominent, irregularly concentric markings or ridges in the lesions (Pl. XXV, C). Unfortunately, the absence of these markings is not absolute proof that the disease is not Fusarium rot; however, they occur more often than not, and the writer has never observed them in lesions produced in any other gladiolus disease. They have been observed consistently on diseased corms of the varieties Fire King, Princeps and Mrs. Francis King.

The lesions are generally somewhat sunken due to the rapid drying and shrinking of the tissues. The advancing margins are usually definite and slightly raised, with active lesions showing a narrow, watersoaked outer area, dark in color, which blends sharply into the healthy tissue. Diseased tissue is hard and in most cases not over 5 to 7 millimeters in thickness. As is true of the hard rot, dry rot and scab, the diseased tissue, especially of less active lesions, can be chipped out with the finger nail, leaving apparently healthy tissue beneath.

The color of the lesions, although generally reddish-brown, varies somewhat with the variety affected. On Mrs. Francis King, hazel[2] is the predominating color; and on Fire King the color range is from burnt sienna to bay, with older lesions almost black.

[2] Designations of colors are according to Ridgway (5).

Fusarium rot is primarily a disease of stored corms, but certain conditions of disease in the field relate directly to the rot which advances during storage. Missing plants in the row may be due to the fact that the corms planted were worthless mummies, their rotted condition being concealed by the apparently healthy husks. Dwarfed, stunted plants, which frequently fail to produce blossoms, result from planting corms so severely diseased that they fail to develop root systems or conducting tissues sufficient to support the developing offspring. Diseased corms commonly decay prematurely in the field, saprophytes, in many if not most cases, playing an important rôle in the rapid disintegration of the tissues.

In two instances the pathogene causing this disease has been isolated from the base of decaying stems, and in one instance from lesions on the roots. In other instances species of *Fusarium* which might have been this fungus have been isolated from stems and roots, but the identities of the fungi were not determined absolutely. It seems certain, however, that the pathogene causing the corm rot may also cause a decay of the stem.

When corms affected with the Fusarium rot are kept under very moist conditions, the lesions commonly become covered with a grayish, mold-like growth of the fungus (Pl. XXV, A). Corms in this condition have been received from growers. Only in relatively few cases, however, has the fungus present on the surface been proved to be the pathogene here described. Under such conditions, common saprophytes are to be expected.

ETIOLOGY

The Fusarium rot of gladiolus is caused by the fungus *Fusarium oxysporum* var. **gladioli** n. var.

SOURCE OF PURE CULTURE

The culture of *Fusarium* referred to in the following descriptions and experiments was isolated from a typically diseased corm of the variety Fire King on February 9, 1925. Tissue plantings were first made, and from a subsequent transfer a strain from a single spore was obtained.

Simultaneously with the studies on which these descriptions are based, cultures of the fungus isolated from other varieties and reisolated from artificially infected corms have been studied sufficiently to eliminate any doubt of the strain from Fire King being pathogenic and being the one most commonly isolated from diseased corms. It is not maintained, however, that other Fusaria may not attack the gladiolus and cause corm rots similar to the one herein described.

MORPHOLOGY

The morphological characters (Figs. 1 and 2) of the organism, as determined from cultures on various media, are as follows:

Microconidia numerous, dominantly 0-septate. Macroconidia gradually attenuated toward both ends, nearly cylindrical in the middle half of their length, somewhat pedicellate, and usually uniformly curved throughout;

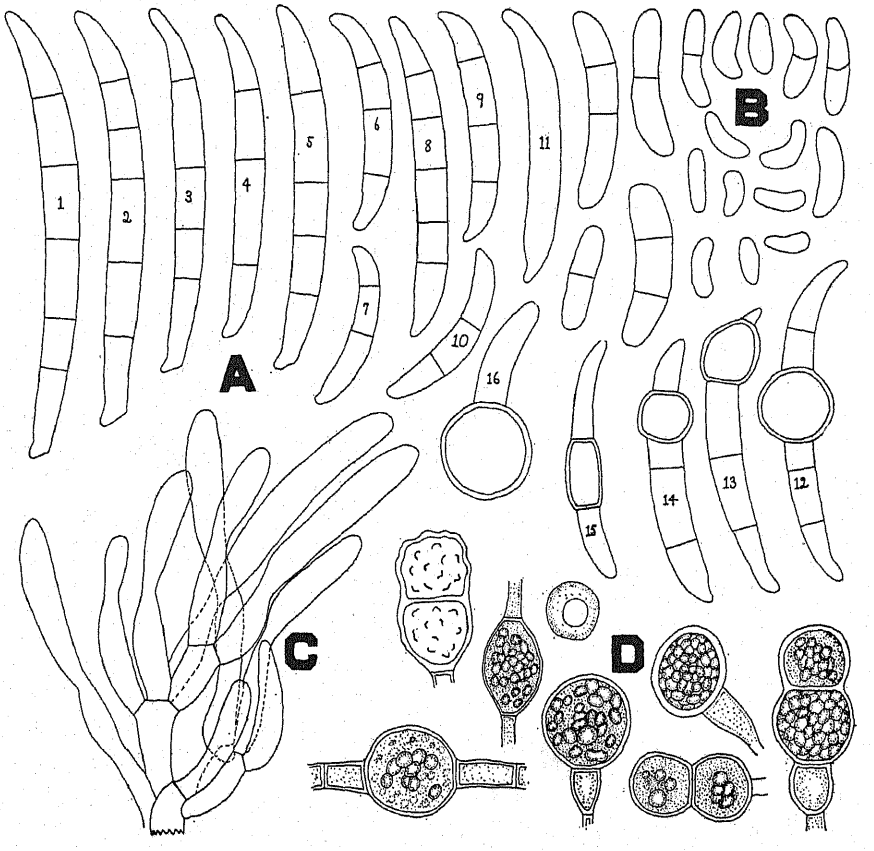

FIG. 1. Spores of *Fusarium oxysporum* var. *gladioli*. A. Macroconidia: 1, 10, from a medium sized sporodochium on hard potato agar after 25 days; 2, from a medium sized sporodochium on potato tuber plug after 39 days; 3, 4, 6, from a small sporodochium on tomato stem after 39 days; 5, 7, 8, from a large sporodochium on corn meal agar after 90 days; 9, 16, from a medium sized sporodochium on cornmeal agar after 38 days; 11, from a small sporodochium on tomato stem after 30 days; 12, 13, 14, 15, from a large sporodochium on tomato stem after 94 days. B, microconidia from culture on hard potato agar after 19 days. C, conidiophore from culture on tomato stem after 8 days. D, chlamydospores from culture on tomato stem after 94 days. Outlined with camera lucida, x 1000.

0-septate up to 98 per cent, 35 x 4.2 (30–42 x 3.7–4.6) μ; 1-septate up to 6 per cent, 20.5 x 3.2 (19–21 x 3.1–3.4) μ; 2-septate up to 10 per cent, 28 x 4.0 (27–30 x 3.9–4.1) μ; 3-septate, dominant, up to 92 per cent, 33.4 x 4.3 (25–41 x 3.5–4.8) μ; 4-septate up to 47 per cent, 39 x 4.4 (33–43 x 3.8–4.8) μ; 5-septate up to 8 per cent, 44 x 4.5 (42–46 x 4.3–4.6) μ; 7-septate, less than 1 per cent, 42 x 4.8 (40–44 x 4.8) μ. Chlamydospores mostly 0-septate, approximately globose and smooth-walled, intercalary or terminal, and commonly present in conidia; in conidia, 0-septate, 9 x 7 (6–14 x 5–10) μ; in mycelium, 0-septate, 11 x 10 (7–17 x 7–16) μ. Aerial mycelium present on most media, typically white and well developed, loosely-cottony, up to 5 mm. in height. Sporodochia up to 2 mm. in diameter, forming in from 7 to 14 days, typically salmon-buff in color. Bluish-black sclerotia present on potato tuber plug and commonly present on other media.

CULTURAL CHARACTERS

Fusarium oxysporum var. *gladioli* was grown on the following media: cornmeal agar, with and without 5 per cent dextrose, in petri dishes, spore dilution cultures; hard potato agar neutral, with and without 5 per cent dextrose, in petri dishes and test tubes; hard potato agar, made acid by the addition of one drop of 50 per cent lactic acid to 10 cubic centimeters of the medium, with and without 5 per cent dextrose, in petri dishes and test tubes; oatmeal agar, in petri dishes and test tubes; lima bean agar, in test tubes; rice, 2 grams in 6 cubic centimeters of water in test tubes; potato tuber cylinders, in test tubes, no water added; sweet clover, raspberry, tomato and potato stems in test tubes, each with 4 cubic centimeters of water added. The vegetable decoctions were prepared according to the formulae given by Sherbakoff (7, p. 105). All agar media contained 3 per cent agar. All media were autoclaved for 45 minutes at 10 pounds pressure, except rice, which was steamed for one hour on three successive days. With the exception of cultures on cornmeal agar, which were spore dilutions, all were initiated by transferring bits of medium containing mycelium from other cultures. The cultures were kept in diffuse daylight at room temperature (approximately 22° C.).

No marked variations or unusual types of growth occurred on any of the media. It was not difficult to maintain the cultures in the state of *Hochkultur* over a period sufficiently long to complete the taxonomic study. The intensity of color production was increased by the addition of lactic acid and of dextrose. The growth rate, as measured by the diameter of the thalli in petri dishes, was slightly retarded by the addition of the lactic acid and slightly increased by the addition of 5 per cent dextrose. Aerial mycelium was present and typically well developed in all cultures with the

exception of those in which the fungus was growing on cornmeal agar. It was 3 to 5 mm. in height, of a loose-cottony appearance becoming slightly more woolly and appressed with age, and dominantly white. In cultures where the aerial mycelium was colored, as for example, hard potato agar with 5 per cent dextrose, the color varied from pinkish vinaceous through dark vinaceous to ox-blood red, representing a diffused color of the stroma. On rice, when 60 days old, the aerial mycelium was dominantly white, ranging to pinkish vinaceous. Sporodochia formation was retarded or entirely prevented by the addition of lactic acid or 5 per cent dextrose. Mature sporodochia were present in cultures on cornmeal agar with 1.5 per cent dextrose in 16 days, and abundant on oat agar in 24 days. They were formed readily and in large numbers on stems of raspberry, tomato, sweet clover, and potato, being mature in about 14 days. They were typically salmon-buff in color.

Only in those cultures in which the fungus was growing in decoctions to which dextrose or lactic acid, or both, had been added, and in cultures on rice and potato tuber plugs, did the substratum become colored. A deep purplish vinaceous color developed in from 5 to 7 days. In about 15 days, in petri dish cultures on potato agar, the color of the substratum became almost black in the center of the dish, grading to the outer edge through bordeaux, dark vinaceous purple and ox-blood red. On rice, when 60 days old, the substratum was dominantly pinkish vinaceous, grading through dark vinaceous to bluish-black deeper in the medium.

In determining the size of the conidia, at least 50 and in some instances 100 spores were measured. The measurements on the various media are as follows:

On cornmeal agar, culture 16 days old; conidia taken from a small sporodochium:
0-septate, 98 per cent, 41.2 x 4.7 (30.0–50.4 x 4.2–6.0) μ.
3-septate, 2 per cent, 42 x 4.8 μ.
On tomato stems, culture 18 days old; conidia taken from a large sporodochium:
0-septate, 92 per cent, 31.5 x 4.6 (25.2–45.6 x 3.6–5.4) μ.
3-septate, 8 per cent, 29.7 x 4.8 (25.2–37.2 x 4.8) μ.
On tomato stems, culture 19 days old; conidia from a large sporodochium:
0-septate, 30 per cent, 35.1 x 4.1 (27.6–49.2 x 3.6–4.8) μ.
2-septate, 4 per cent, 33.6 x 3.6 (31.2–36.0 x 3.6) μ.
3-septate, 52 per cent, 35.1 x 4.0 (27.0–48.0 x 3.0–4.8) μ.
4-septate, 14 per cent, 43.0 x 4.1 (38.4–50.4 x 3.6–4.8) μ.
On potato stem, culture 21 days old; conidia from a medium sized sporodochium:
0-septate, 34 per cent, 29.5 x 3.6 (21.6–36.0 x 3.0–4.2) μ.
1-septate, 2 per cent, 19.2 x 3.0 μ.
2-septate, 10 per cent, 26.2 x 3.7 (19.2–31.2 x 3.6–3.9) μ.
3-septate, 54 per cent, 29.8 x 3.9 (24.0–36.0 x 3.0–4.8) μ.
On potato tuber plug, culture 21 days old; conidia taken from a small sporodochium:
0-septate, 30 per cent, 32.8 x 4.2 (28.8–45.6 x 3.6–4.8) μ.

3 septate, 44 per cent, 30.4 x 4.3 (20.4–38.4 x 3.0–4.8) μ.
4-septate, 24 per cent, 35.0 x 4.5 (30.0–39.6 x 3.6–4.8) μ.
5-septate, 2 per cent, 37.2 x 4.2 μ.

On sweet clover stems, culture 22 days old; conidia taken from a small sporodochium:

0-septate, 7 per cent, 27.8 x 4.0 (20.0–36.0 x 3.3–4.2) μ.
3-septate, 83 per cent, 32.6 x 4.3 (20.0–42.0 x 3.6–4.8) μ.
4-septate, 10 per cent, 37.4 x 4.2 (33.6–40.8 x 3.6–4.8) μ.

On locust stems, culture 22 days old; conidia taken from a small sporodochium:

1-septate, 4 per cent, 24.0 x 3.3 (20.4–27.6 x 3.0–3.6) μ.
2-septate, 2 per cent, 31.2 x 3.6 μ.
3-septate, 86 per cent, 31.3–4.1 (21.6–39.6 x 3.0–4.8) μ.
4-septate, 8 per cent, 34.8 x 4.0 (32.4–37.2 x 3.6–4.5) μ.

On hard oat agar, culture 23 days old; conidia from a large sporodochium:

3-septate, 54 per cent, 34.7 x 4.4 (26.4–46.2 x 3.6–4.8) μ.
4-septate, 44 per cent, 41.5 x 4.4 (34.8–48.0 x 4.2–4.8) μ.
5-septate, 2 per cent, 45.6 x 4.2 μ.

On hard oat agar, culture 25 days old; conidia taken from a medium sized sporodochium:

0-septate, 2 per cent, 39.6 x 3.6 μ.
2-septate, 2 per cent, 32.4 x 4.8 μ.
3-septate, 48 per cent, 35.3 x 4.5 (24.0–45.6 x 3.6–4.8) μ.
4-septate, 40 per cent, 44.2 x 4.5 (36.0–56.4 x 4.2–4.8) μ.
5-septate, 8 per cent, 47.1 x 4.4 (44.4–49.2 x 4.2–4.8) μ.

On hard oat agar, culture 25 days old;

(a) Conidia taken from a medium sized sporodochium:
3-septate, 71 per cent, 34.8 x 4.3 (25.2–44.4 x 3.0–4.8) μ.
4-septate, 28 per cent, 39.1 x 4.5 (33.6–48.0 x 3.6–4.8) μ.
5-septate, 1 per cent, 55.8 x 4.8 μ.

(b) Chlamydospores in mycelium:
0-septate, 100 per cent, 10.6 x 11.0 (6.0–14.4 x 7.2–15.6) μ.

On hard potato agar, culture 36 days old;

(a) Conidia taken from a medium sized sporodochium:
1-septate, 6 per cent, 20.8 x 3.2 (19.2–21.6 x 3.0–3.6) μ.
2-septate, 2 per cent, 25.2 x 3.6 μ.
3-septate, 62 per cent, 33.8 x 4.1 (27.6–39.6 x 3.6–4.8) μ.
4-septate, 30 per cent, 38.4 x 4.2 (36.0–45.6 x 3.9–4.8) μ.

(b) Chlamydospores in conidia:
0-septate, 98 per cent, 8.7 x 6.8 (6.0–10.8 x 4.8–8.4) μ.
1-septate, 2 per cent, 9.6 x 6.0 μ.

On cornmeal agar, culture 36 days old; conidia taken from a large sporodochium:

2-septate, 1 per cent, 19.2–3.6 μ.
3-septate, 55 per cent, 33.2–4.2 (21.6–44.4 x 3.0–4.8) μ.
4-septate, 38 per cent, 38.1 x 4.4 (32.4–48.0 x 3.6–4.8) μ.
5-septate, 6 per cent, 40.8 x 4.7 (38.4–45.6 x 4.2–4.8) μ.

On hard oat agar, culture 38 days old; conidia taken from a large sporodochium:

3-septate, 74 per cent, 32.8 x 4.3 (26.4–39.6 x 3.6–4.8) μ.
4-septate, 18 per cent, 39.6 x 4.1 (33.6–44.4 x 3.6–4.2) μ.
5-septate, 8 per cent, 47.1 x 3.7 (42.0–52.8 x 3.6–4.2) μ.

On cornmeal agar, culture 38 days old;

(a) Conidia taken from a medium sized sporodochium:

2-septate, 2 per cent, 27.6–4.2 μ.
3-septate, 58 per cent, 34.1 x 4.2 (24.0–38.4 x 3.3–4.8) μ.
4-septate, 32 per cent, 38.3 x 4.5 (32.4–48.0 x 3.6–4.8) μ.
5-septate, 8 per cent, 41.1 x 4.7 (38.4–43.2 x 4.5–4.8) μ.

(b) Chlamydospores in conidia:

0-septate, 100 per cent, 9.7 x 8.9 (4.8–15.6 x 4.8–14.4) μ.

On potato tuber plug, culture 39 days old; conidia taken from a medium sized sporodochium:

0-septate, 2 per cent, 38.4 x 4.8 μ.
2-septate, 6 per cent, 21.6 x 3.8 (20.4–22.8 x 3.6–4.2) μ.
3-septate, 78 per cent, 29.3 x 4.3 (20.4–38.4 x 3.6–4.8) μ.
4-septate, 14 per cent, 32.7 x 4.5 (24.0–39.6 x 4.2–4.8) μ.

On tomato stem, culture 39 days old;

(a) Conidia from a small sporodochium:

0-septate, 1 per cent, 38.4 x 4.2 μ.
1-septate, 1 per cent, 15.6 x 3.0 μ.
2-septate, 3 per cent, 32.8 x 4.1 (27.0–38.4 x 3.6–4.5) μ.
3-septate, 83 per cent, 36.8 x 4.4 (19.2–44.4 x 3.6–4.8) μ.
4-septate, 11 per cent, 41.1 x 4.4 (34.8–51.6 x 4.2–4.8) μ.
5-septate, 1 per cent, 40.8 x 4.8 μ.

(b) Chlamydospores in conidia:

0-septate, 100 per cent, 8.3 x 6.6 (6.0–11.4 x 4.8–7.8) μ.

On cornmeal agar, culture 90 days old; conidia taken from a medium sized sporodochium:

1-septate, 1 per cent, 22.8 x 3.6 μ.
2-septate, 2 per cent, 21.0 x 3.9 (19.2–22.8 x 3.6–4.2) μ.
3-septate, 40 per cent, 32.9 x 4.5 (24.0–39.6 x 3.6–5.4) μ.
4-septate, 47 per cent, 36.7 x 4.7 (30.0–50.4 x 4.2–5.4) μ.
5-septate, 8 per cent, 40.8 x 4.7 (34.8–44.4 x 4.2–4.8) μ.
7-septate, 2 per cent, 42.0 x 4.8 (39.6–44.4 x 4.8) μ.

On hard oat agar, culture 92 days old; chlamydospores in conidia:

0-septate, 100 per cent, 9.6 x 6.6 (6.0–16.8 x 4.5–9.6) μ.

On tomato stem, culture 94 days old; chlamydospores in mycelium:

0-septate, 92 per cent, 12.0 x 10.7 (7.8–19.2 x 7.2–15.6) μ.
1-septate, 8 per cent, 19.2 x 12.6 μ.

On locust stems, culture 105 days old; conidia taken from a medium sized sporodochium:

0-septate, 4 per cent, 33.6 x 4.1 (30.0–37.2 x 3.6–4.5) μ.
3-septate, 92 per cent, 32.3 x 3.8 (21.5–44.5 x 3.0–4.8) μ.
4-septate, 4 per cent, 41.2 x 4.2 μ.

Average of the above measurements:[3]

Conidia: 0-septate, 30 per cent, 34.8 x 4.2 (30.0–41.6 x 3.7–4.6) μ.
1-septate, 3 per cent, 20.5 x 3.2 (19.4–21.4 x 3.1–3.4) μ.
2-septate, 3 per cent, 28.4 x 4.0 (26.8–29.9 x 3.9–4.1) μ.
3-septate, 58 per cent, 33.4 x 4.3 (25.0–41.0 x 3.5–4.8) μ.
4-septate, 26 per cent, 38.6 x 4.4 (33.0–42.7 x 3.8–4.8) μ.

[3] Percentages calculated on the basis of cultures in which the indicated conidia were found—not on total number of cultures studied.

5-septate, 5 per cent, 44.0 x 4.5 (41.9–46.1 x 4.3–4.6) μ.
7-septate, less than 1 per cent, 42.0 x 4.8 (39.6–44.4 x 4.8) μ.
Chlamydospores:
(a) in conidia, 0-septate, 99 per cent, 9.1 x 7.2 (5.7–13.6 x 4.7–10.5) μ.
(b) in mycelium, 0-septate, 96 per cent, 11.3 x 10.9 (6.9–16.8 x 7.2–15.6) μ.

From a comparison with descriptions by Wollenweber (9), Sherbakoff (7) and Wollenweber *et al.* (10), it is evident that this fungus (Figs. 1 and 2) falls in the section Elegans of the genus *Fusarium*, and within that group lies so close to *Fusarium oxysporum* Schlecht. emend. Wr. (6, 8) as to be considered a variety of this well-known species. It differs from *F. oxysporum* in having slightly longer macroconidia, a higher percentage of 4-septate spores, somewhat weaker development of the "foot" (Figs. 1 and 2), and especially in its parasitism (see under pathogenicity, below). For these reasons it would seem that the gladiolus fungus should be made at least a new variety, the alternative being to make it a new species.[4]

PATHOGENICITY

The ability of *Fusarium oxysporum* var. *gladioli* to infect the gladiolus and produce lesions on corms typical of those from which the fungus has been isolated many times has been established through numerous experiments both in the field and under greenhouse conditions. Infection takes place when the fungus is added to the soil before the corms are planted, when the corms are dipped in suspensions of spores from a pure culture and either planted in soil or else held in moist chambers in the laboratory, and when inoculations are made with the pathogene into newly developing corms from healthy corms planted in clean soil.

On March 28, 1916, 12 plants, variety Meadowvale, growing in the greenhouse bench were inoculated when they were from 8 to 10 inches in height. The soil was removed from about the corms and a bit of potato agar carrying the fungus was pressed against each of the uninjured corms. The inoculum was then covered with cotton saturated with water, and the soil replaced. When an examination was made on April 12, at which time the experiment was discontinued, it was found that lesions typical of Fusarium rot were present on 8 of the 12 corms. Check plants remained healthy. The fungus was reisolated from these lesions and its identity determined.

In the summer of 1921 infection was obtained by inoculating corms of the varieties Princeps, Augusta, Peace, Mrs. Francis King, America, Fire King, Meadowvale, and three unknown varieties, as well as Primulinus

[4] Assistance in the determination of the fungus was given by Dr. C. D. Sherbakoff, who concurred with the writer in his decision that the fungus should be made a variety of *F. oxysporum* Schlecht. emend. Wr.

Hybrids. These were growing in an experimental garden in soil in which no gladioli had ever been grown. The parent corms were planted June 1, and the corms developing from them were inoculated during August when they were approximately one inch in diameter. Of the 15 corms of each

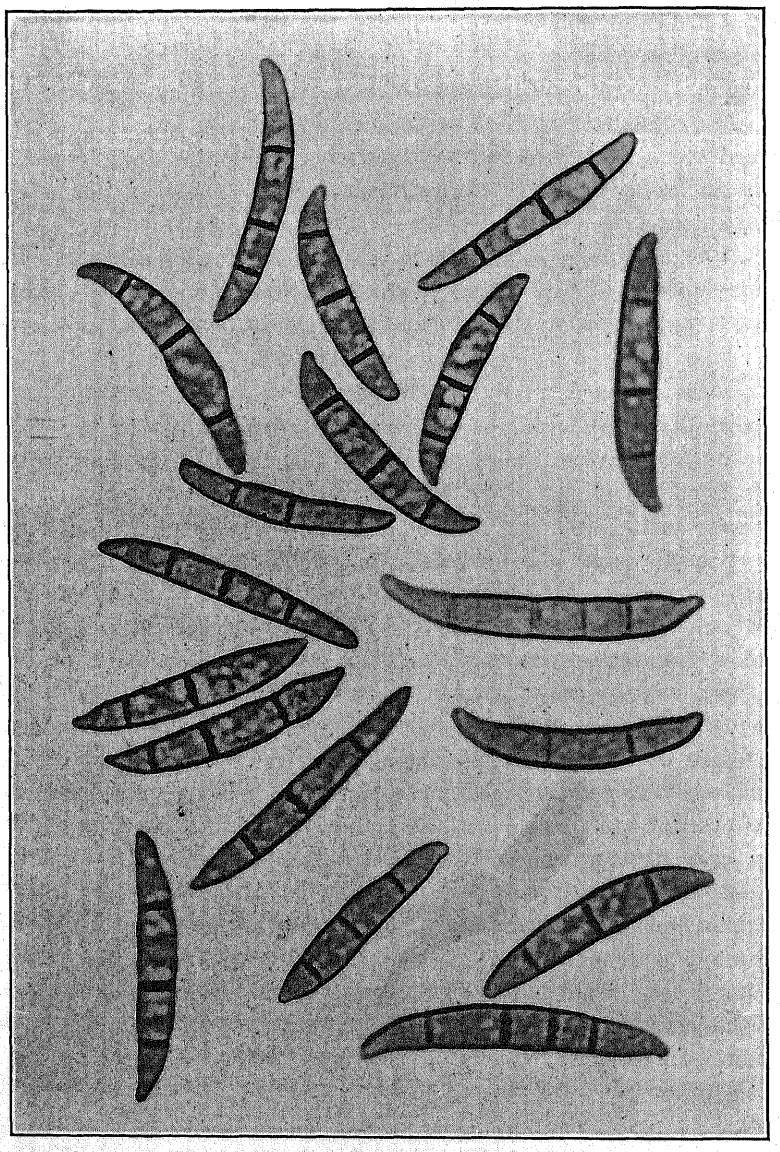

FIG. 2. Macroconidia of *Fusarium oxysporum* var. *gladioli*, from medium sized sporodochium on sweet clover stem after 63 days. Photomicrograph, x 800.

variety inoculated, 5 were injured by making several cuts through the sheathing leaf bases into the body of the corms, and the remainder uninjured. The inoculum was taken from a two months' old culture of the fungus on sterilized wheat kernels. The method involved pulling away the soil from the base of the plant and pressing about six kernels of wheat carrying the fungus against the injured or uninjured surface. The inoculum was then covered with cotton saturated with water and the soil replaced. Five plants of each lot were given similar treatment with the exception that the fungus was omitted. No evidences of disease, as indicated on plant parts above ground, developed prior to harvest in October, and the corms were not examined until the following January. Infection was obtained on all varieties, and the checks were found to be sound. The percentages of infection were 90 and 68, respectively, of those injured and uninjured.

That infection may be obtained under artificial conditions was proved by the following experiment. Fifty large corms of the variety Mrs. Francis King were selected as being sound, no lesions being evident from a critical examination following the removal of the husks. They were then placed in 50 per cent alcohol for 3 minutes, in 1–1,000 mercuric chloride for 15 minutes, given five washings in distilled water, and then placed on moistened filter paper in moist chambers, 240 by 80 mm., which had been sterilized previously. Two kinds of inoculum were used. The first consisted of bits of medium bearing the fungus, taken from a 9-days old culture on potato agar; the second, a suspension of spores from sporodochia produced on tomato stems. In the first case the method consisted in pressing the inoculum against the uninjured surface of the corms. In using the second type of inoculum, somewhat less than one cubic centimeter of the spore suspension was dropped into the depressions occurring naturally on the upper surfaces of the corms. Each inoculated lot consisted of 20 corms, and 10 corms were carried as checks. The moist chambers were kept in a constant temperature chamber at 25° C., and water was added as needed to keep the filter papers saturated.

The inoculations were made on April 30, 1921. On June 10, lesions measuring from one-half to one inch in diameter were present on all the inoculated corms. By July 15, the rot involved from one-half to the entire surface of the corms, and a mold-like growth of the fungus covered the lesions. This growth was powdery in appearance, due to the large numbers of spores, and numerous sporodochia also were present. The checks remained free from decay.

Several other experiments similar to the above have been conducted at various times and have always yielded a high percentage of infection. Some of the earlier experiments gave results indicating that varieties with dark

colored corms were more resistant than those with white and light yellow corms, but later experiments failed to substantiate this possibility.

Infection has been secured by inoculating uninjured stems at a distance of 8 to 12 centimeters above the surface of the soil. In all cases the inoculum was covered with cotton, which was kept moist by adding water from time to time during the first week following inoculation. In about three weeks the rot had advanced down the stem to below the soil line and had progressed above the point of inoculation about 5 centimeters. The foliage became yellow and died prematurely.

No infection with *F. oxysporum* var. *gladioli* has been obtained in several trials on living stems of tomato, pepper, egg-plant and potato. In one experiment, negative results were obtained on potato plants under conditions which yielded infection with a culture of *F. oxysporum* obtained from Dr. C. D. Sherbakoff. The inoculations were made by introducing the fungi growing on wheat kernels into longitudinal slits in potato stems at about the surface of the soil. The plants were growing in the greenhouse and were from 6 to 8 inches in height when inoculated. The stems at the point of inoculation were wrapped with moistened cotton which was not removed throughout the duration of the experiment. Twenty stems were inoculated with each of the two fungi. The check consisted of the same number of stems given similar treatment, except that no fungous growth was introduced into the stems along with the wheat kernels.

The inoculations were made on August 15, 1925. On September 15 it was found that 15 of the 20 stems inoculated with *F. oxysporum* were infected, as evidenced by the discoloration of the vascular tissues for a distance of about 2.5 centimeters below and 6.5 centimeters above the point of inoculation. No pronounced wilting was observed. Tissue plantings were made from these stems and the fungus obtained in culture. In the case of the gladiolus fungus, a very slight discoloration, less than 1.5 centimeter in total extent, occurred at the point of inoculation, but there were no indications that the fungus was pathogenic to potatoes. It would seem, then, that the parasitism of *Fusarium oxysporum* var. *gladioli* is not identical with that of *Fusarium oxysporum* from potatoes.

TEMPERATURE RELATIONS

Cultures of *F. oxysporum* var. *gladioli* were established by transferring bits of a solid medium carrying mycelium to the center of petri dishes of equal size containing equivalent amounts of hard potato agar with 1 per cent dextrose. The medium was tested electrometrically and found to have a pH value of 7. After incubating all of the cultures for 24 hours at 20° C., they were divided into lots of four dishes each and each lot held for 10

hours at the particular temperature at which it was to be kept throughout the experiment. This permitted the fungus to establish itself in the medium in the petri dish and eliminated errors that might have arisen from failure to allow sufficient time for temperature adjustments before making the initial measurements. The diameters of the thalli were then recorded and the rate and amount of subsequent growth determined by measuring the increases over the original diameters. Four cultures were placed in each of a series of temperature chambers with the following temperatures: 0°, 5°,

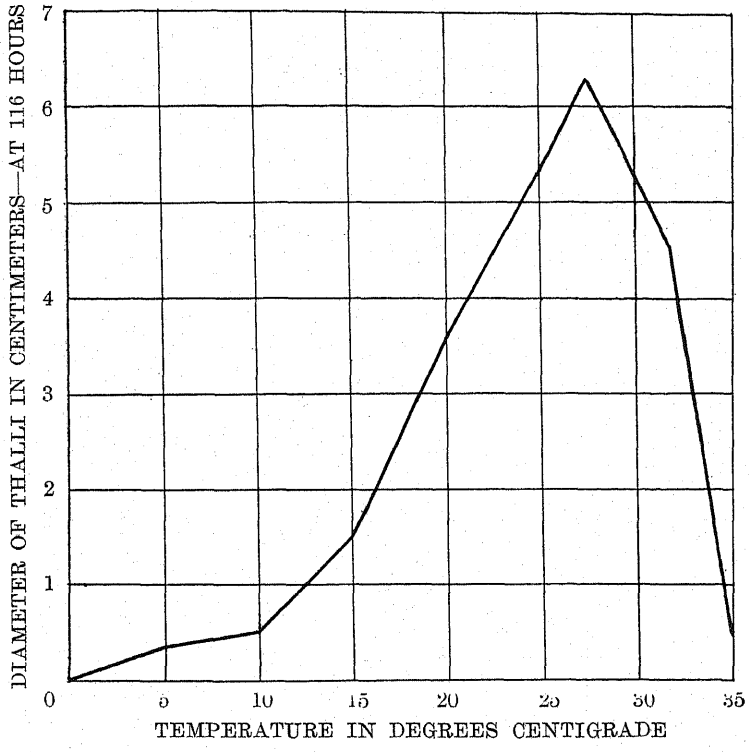

FIG. 3. Relation of temperature to growth of *Fusarium oxysporum* var. *gladioli* on hard potato agar with 1 per cent dextrose in petri dishes.

10°, 15°, 20°, 25°, 27.5°, 30°, 32° and 35° C. The diameters of the thalli at 116 hours were taken as a criterion of comparison, the results being represented graphically in figure 3. There is a gradual increase in rate of growth up to 25° with optimum near 27.5°, and a rapid drop beyond 30° C.

CONTROL

No experiments in the control of this particular disease have been attempted. From what is known regarding the nature of the disease, its

cause and the life history of the pathogene, it would seem that the problem of control would not differ greatly from that of the hard rot and dry rot diseases. For the latter two, it has been found that sorting, with the elimination of diseased corms, combined with a four or five years' rotation and proper handling of the corms at harvest and during storage are usually sufficient to keep the losses at a minimum. To be properly handled, the corms should be dug when they can be dried in the open for a day or two before being placed in storage. As little soil as possible, either adhering to or accompanying the corms, should be taken indoors. The old roots and adhering soil should be removed early during the storage period.

Throughout the first month the storage house should be given maximum ventilation to carry off the moisture and to facilitate drying. As early as possible in sections with cold winters, as in New York State, the temperature should be dropped to about 35° F., where it should be held throughout the winter.

Besides maintaining a low temperature, any practices which lower the humidity of the air about the corms is to be recommended. In instances where their use is feasible, trays with slatted rather than solid bottoms are preferred, and these are best stacked in racks which will permit the movement of air on all sides of them. The corms should not be piled deeper than 4 inches in the trays, and where possible should be stirred several times during the winter. Sorting, with the elimination of diseased corms, should be done late in the spring, since at this time the advanced stage of decay makes it easier to pick out those that are affected.

SUMMARY

Fusarium rot is an important disease of gladiolus corms in the United States. The disease probably occurs to some extent in all collections of gladioli of any considerable size. A description of the disease is given. Corms become infected in the field, and the rot advances during storage. Diseased corms in storage may be reduced to worthless mummies under favorable conditions for the disease, and the planting of infected corms frequently results in the death of plants in the field during mid-season.

The pathogene, *Fusarium oxysporum* var. *gladioli* n. var., belongs to the section Elegans, differing from *F. oxysporum* Schlecht. emend. Wr. in certain morphological characters and in its parasitism. Its morphological and cultural characters are recorded.

On potato agar, the fungus grows over a range of from about 5° to 35° C. Optimum growth takes place at about 27.5° C.

Infection was readily obtained on uninjured corms in the laboratory and on those growing in the greenhouse and experimental gardens.

Although no experiments on control were attempted, it is believed that sorting, with the elimination of diseased corms, together with crop rotation and the proper handling of corms at harvest and during storage, will greatly reduce losses from the disease.

CORNELL UNIVERSITY,
ITHACA, N. Y.

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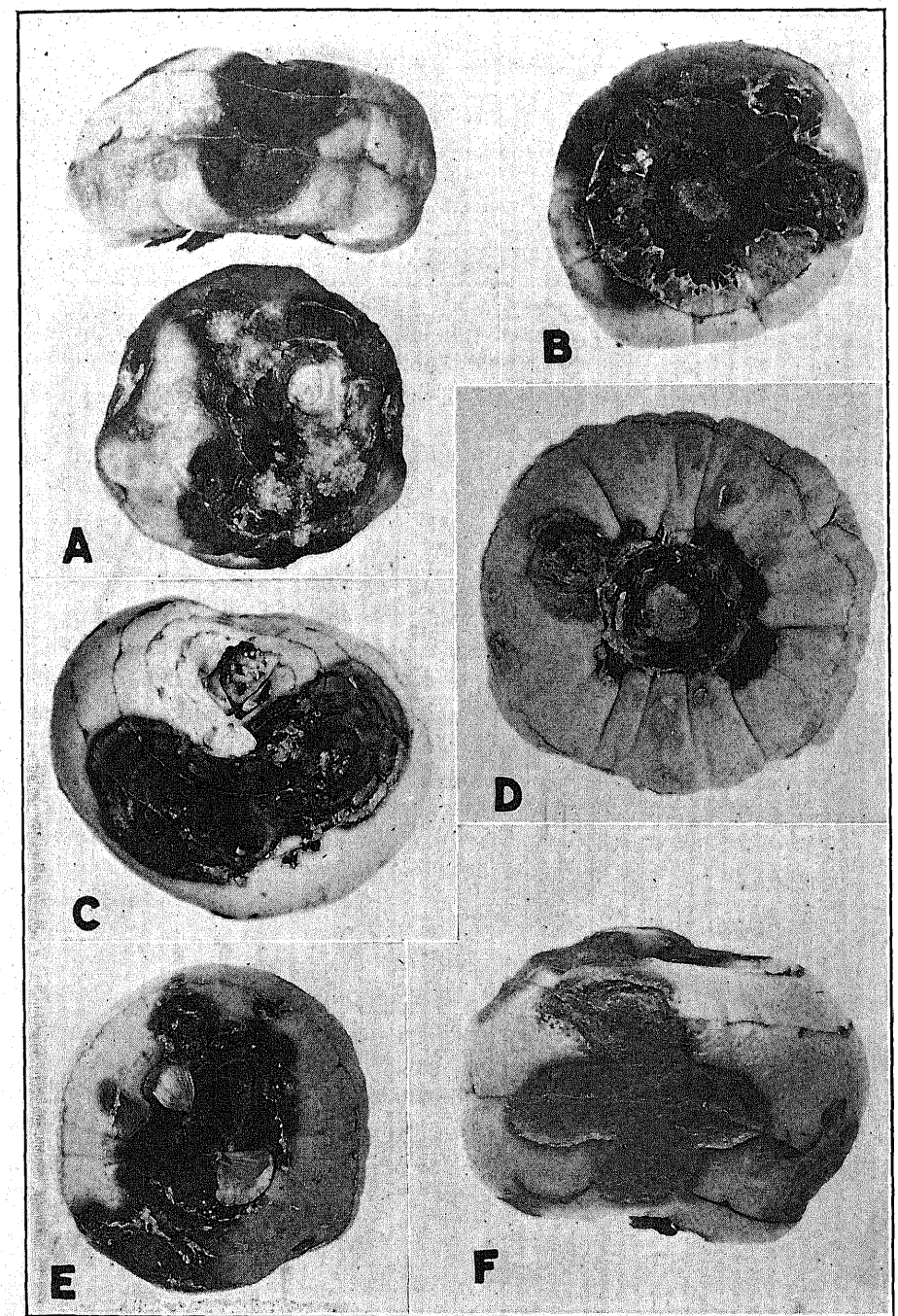
EXPLANATION OF PLATE XXV

Corms affected with *Fusarium oxysporum* var. *gladioli*. A. Variety Princeps, natural infection. B, E. Variety Fire King, natural infection. C. Variety Mrs. Francis King inoculated with pure culture of fungus isolated from Fire King and kept in moist chamber at 24° C. Photographed after 30 days. D. Variety America, natural infection. F. Unknown variety inoculated with pure culture of fungus isolated from Princeps and kept in moist chamber at 24° C. Photographed after 28 days.

All natural size with the exception of F which is x $1\frac{2}{3}$.


A
B
C
D
E
F

SOME FACTORS AFFECTING THE PATHOGENICITY OF ACTINOMYCES SCABIES[1]

G. B. SANFORD[2]

INTRODUCTION

Common scab of potato, *Solanum tuberosum,* caused by *Actinomyces scabies* (Thaxter) Güssow, is prevalent and destructive. All attempts effectively to control the disease have failed. The potato scab pathogene is normally a soil saprophyte, and its activities are therefore influenced by other soil microorganisms. There may be metabiotic or antibiotic relations between these organisms, and these relations, as well as the soil flora, may be altered when one or more of the physical or chemical factors of the soil are changed, but the degree of this modification and its effect on scab development is not well understood.

A review of the work done for the control of potato scab previous to 1914 has been given by Lutman and Cunningham (9). Drechsler (3) has studied the morphology of *Actinomyces,* and Waksman (17) the physiology. Others have studied various methods of reducing scab. In addition to tuber disinfection, the principal preventive measure used has been to attempt to increase the acidity of the soil. This appears to have serious economic and other limitations on many potato soils, especially those having a high initial pH value and which are highly buffered. The plowing down of green crops, particularly rye, often is recommended as a control measure for potato scab, as the decomposition of organic matter thus put into the soil is assumed to increase soil acidity.

OBJECTS OF THE INVESTIGATION

The objects of this investigation were: to study the effect of temperature, moisture, oxygen supply, soil reaction, and stimulatory substances on the germination, growth and pathogenicity of *A. scabies*; and to throw additional light on the nature of the control said to be obtained by plowing under a green crop of rye.

[1] Published with the approval of the Director as Paper No. 618 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Grateful acknowledgment is made to Professor E. C. Stakman for his assistance and helpful criticism during this study.

METHODS

A synthetic dextrose-nitrate substrate was used for all studies, except where otherwise stated, the composition being: K_2HPO_4, 1 gm; $MgSO_4$, 0.5 gm; KCl, 0.5 gm; $FeSO_4$, trace; $NaNO_3$, 2 gm; dextrose 30 gm; agar 18 gm; distilled water 1000 cc. Agar slants in loosely plugged test tubes were found preferable to petri dish cultures. It was found advisable to add the spores[3] in suspension to the surface of the solid medium. Other methods will be described when necessary. A strain of *A. scabies* of tested pathogenicity was labelled "B" and used in all studies, except where otherwise stated.

EXPERIMENTAL RESULTS

Temperature Relations

The effect of temperature on spore germination has received attention by Shapovalov (15) and others, but a study of the influence of temperature on the pathogenicity of the strain of *A. scabies* used in these experiments seemed advisable.

Effect on growth and pathogenicity. Three strains of *A. scabies* (A, B and C) were used. Strains B and C were obtanied from S. A. Waksman, Rutgers College, N. J., and strain A was isolated by the writer and tested for pathogenicity. Duplicate cultures on nutrient agar slants were kept at the following temperatures: 7–8°, 10°, 13°, 16°, 19°, 22°, 24°, 26–27°, 29–30°, 32°, 35°, 37–38° C., and the growth recorded at 18-, 36-, 60-, 80- and 160-hour intervals. This experiment was repeated three times. All three strains reacted alike. The data for strain B are given in table 1.

TABLE 1.—*The effect of temperature on the growth of Actinomyces scabies, strain B*

Time in hours	Temperature in degrees Centigrade											
	7–8	10	13	16	19	22	24	26–27	29–30	32	35	37–38
	Rate of growth[a]											
18	0	0	0	0	0	1	2	4	5	4	2	0
36	0	0	0	0	1	3	4	4	6	5	3	0
60	0	0	0.5	1	4	5	6	7	8	6	3	1
80	0	trace	2	3	6	7	10[b]	12[c]	10	6	2	2
160	0	5	8	9	10	11	12[c]	12[c]	10	5	2	1

[a] Estimated value given to the relative progress of cultures after germination.
[b] Darkening.
[c] Dark.

[3] "Spores" is used in the widest sense because both conidia and mycelial segments function as reproductive structures.

It is evident from results that the range of temperature for growth increases with the time, and that germination and maturity take place sooner at the higher temperatures, with a corresponding decrease in vegetative growth. For instance, after 18 hours growth occurs between 22° and 35° C., while after 160 hours the range of temperature for growth extends from 10° to 38° C. Although good growth and maturity are attained at the lower temperatures in a longer time, the optimum point for greatest vegetative development and earliest maturity is 26–27° C. These results agree reasonably well with those of Lutman and Cunningham (9) who reported the optimum temperature to be 25° C.

Jones, McKinney and Fellows (6) stated that scab developed best at about 22° C., and that the amount decreased toward 11° and 30.5° C., where scab became negligible, as did also the yield of the tubers. They also suggested that the immediate effect of high temperature is more closely correlated with tuber development than with the growth of the pathogene. Scant growth of individual tubers, however, would not always explain the decrease of scab, because large scab pustules frequently form both on small and large tubers grown under field conditions. High temperatures may retard or prevent the growth of the scab pustule, subsequent to infection, but our knowledge of the process of tuber infection and scab development is insufficient to permit of conclusions.

The writer (14) obtained a maximum amount of scab in soils held at constant moisture content and temperatures of 14°, 17°, and 25° C.; and there was every indication that much scab would develop at at least two degrees lower or higher than 14° and 23° respectively. These results, with those of the present study, indicate that the growth of the pathogene in pure cultures, within the range of temperatures tested, is fairly well correlated with the amount of scab which develops. It might be concluded that temperatures lower than 12° C. will largely prevent scab development under field conditions and that periodic fluctuations between this point and 25° C. would not greatly affect the amount of scab. However, with various biologic and other factors, and a critical period for infection, temperature may prove to be more important in the field than it appeared to be under the controlled soil conditions tested.

Effect on viability. To determine the thermal death point of *A. scabies,* spores from a mature culture were soaked in water for thirty minutes and a very small portion of the spore suspension was put into 5 cc. of sterile water at the temperature under test. At ten- and twenty-minute intervals, portions were withdrawn and spread on cold agar plates to incubate. Repeated tests showed that spores germinated after having been exposed for twenty minutes at 83° but not after exposure at 85° C. They failed to germinate when exposed for ten minutes at temperatures higher than 90° C.

Since the spores of the scab organism resisted such high temperatures, it was decided to test the efficacy of the hot formaldehyde treatment. Experiment showed that scab spores on tubers were killed when immersed for two minutes in the hot formaldehyde solution (2 pints to 30 gallons of water) at 53° C. It also seemed desirable to test the efficacy of the acidulated mercuric chloride treatment for killing spores, because, if successful, its use would obviate the difficulty of the heating required with the formaldehyde treatment. The formula found effective by Cunningham (1) for killing sclerotia of *Rhizoctonia solani* was used. This method consists of soaking the tuber in a 1–1000 solution of mercuric chloride in water, to which has been added 5 cc. of commercial hydrochloric acid per liter. The tubers are kept moist for 24 hours after soaking. Experiments by the writer showed that this method was equally effective in killing spores of *A. scabies* and Rhizoctonia sclerotia.

Oxygen Requirements

Experiments were made to obtain more information on the relation of oxygen to the germination of the spores, the vegetative growth and fructification of *A. scabies,* because the pathogenicity of this soil fungus and the development of scab may be modified by the available oxygen in the soil.

Effect on germination and growth. A simple preliminary experiment suggested the strongly aerobic qualities of *A. scabies.* Six agar slants were inoculated with a spore suspension of the organism. Three tubes were sealed, and three were loosely plugged with cotton. The very faint growth which appeared after 30 hours in the sealed tubes was a striking contrast to the abundant development in those which were loosely plugged. No further increase occurred in the sealed tubes during 30 days. Apparently the available supply of oxygen had been exhausted during the first 30 hours. Another experiment, using the Büchner tube apparatus with alkalinated pyrogallic acid, demonstrated that the spores of *A. scabies* do not germinate in the absence of oxygen.

The relation of pure oxygen and nitrogen gas to the germination of the spores of the scab organism was also tested. These gases were allowed to displace the air in culture flasks, and then the flasks were sealed. No spores had germinated in 30 days in either gas, but, when exposed again to air, those which had been in pure nitrogen germinated and grew well. The spores which had been in oxygen were dead.

The effect of very limited and measurable amounts of oxygen on spore germination was also studied. Partial pressures, ranging from 0 to 29 inches, were obtained in test tubes containing agar slants which had been evenly inoculated with a spore suspension. Fresh inoculum was spread on the surface of the slants at the time the experiment was begun. The tubes

were fitted with rubber stoppers and glass tubes, and then sealed by heat after the air had been exhausted. By repeated experiment the results shown in table 2 were obtained. At zero partial pressure the spores required all the oxygen present in a 25 cc.-test tube to germinate and produce a very scant growth, while at a partial pressure of about 29 inches there was no visible sign of germination when examined microscopically. At a partial pressure of 28 inches short germ tubes appeared on some spores, but most of them did not germinate. The growth was very meager at a partial pressure of 27 inches. At partial pressures 25, 20, 15, 10, and 5 inches, a gradual increase in surface growth was evident over that obtained at a partial pressure of 27 inches, and increasingly higher percentages of spores also germinated at the lower partial pressures. The growth in the control tubes, which were loosely plugged with cotton, was in striking contrast to that at a partial pressure of 0 inches.

Effect on maturity. The relation of a limited amount of oxygen to vegetative growth, fructification, and especially to maturity (the darkening process of aerial hyphae) of *A. scabies* was investigated. Three inoculated petri dish cultures were placed in a large desiccator, a quantity of alkalinated pyrogallic acid calculated to absorb nearly all the oxygen was put at the base, and the cover sealed. White vegetative growth became evident in the control cultures in 24 hours and continued to increase, and on the fourth day the hyphae darkened rapidly. The test cultures grew as well as the controls up to the third day, but they failed to darken or to develop further during eleven days. When air was admitted, the retarded cultures grew quickly. In twenty hours they had darkened very noticeably, and by forty-eight hours were almost as dark as the controls. No detached spores and segments of mycelium were evident when the retarded cultures were examined on the eleventh day, but a later examination of the mature cultures revealed both the spores and segments common to ripe hyphae.

Relation of quantity of oxygen to partial pressure. While observing the germination and growth of *A. scabies* at the high partial pressures, it was suggested that the fungus might not be able to obtain the small quantity of oxygen left in the test tube; that is, the quantity of oxygen and not the partial pressure becomes the limiting factor to growth. No data on this point were found in the literature. Accordingly, a partial pressure of about 29 inches was obtained in two 25 cc. test tubes containing inoculated agar slants, and also in two 11,550 cc. bottles each containing one inoculated agar slant. The partial pressure was tested both in the large and small containers after ten days and found to have remained at about 29 inches. At this time, meager growth, similar to what had occurred in the test tubes at a partial pressure of 10–5 inches (see table 2) could be seen on the slant contained in each of the large bottles. The spores in the small test tubes at

in a state of nature. Other factors than acidity of course may determine resistance in some of these plants.

LABORATORY OF PLANT PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

FUSARIUM ROT OF GLADIOLUS CORMS

L. M. MASSEY[1]

The two most important fungous diseases of gladiolus corms are those to which the names hard rot (2) and dry rot (3) have been given. These are to be found in practically all collections of any size, whereas a third disease, Fusarium rot (4), is sometimes present in sufficient amount to be given equal rank in importance.

Fusarium rot was observed as early as 1912. Although it received casual attention over a period of several years, no special investigation of it was undertaken by the writer until 1916. At about this time what seemed to be the same fungus was isolated from so many diseased corms sent to the Department of Plant Pathology at the New York State College of Agriculture that it was considered advisable to make a more intensive study of the disease and its cause.

Diseased corms have been received from a sufficiently large number of growers in New York to warrant the conclusion that Fusarium rot is of general occurrence in this state. Corms affected with the disease also have been received from Maryland, District of Columbia, New Jersey, Ohio, Indiana, Michigan, Wisconsin, Minnesota, California, and Ontario, Canada. Whether or not the plants were grown by the growers who sent the diseased material has not always been clear. However, with the general practice of growers buying relatively large quantities of corms from one another, it seems probable that diseased corms have been distributed throughout the United States and that the disease occurs in some degree in all areas suitable to the growth and parasitism of the fungus.

No instances of a high percentage of diseased corms in large collections, as is common in the case of both the hard rot and the dry rot, have come to the attention of the writer. In these latter diseases, especially hard rot, it is not uncommon to find over 90 per cent diseased corms in collections numbering several hundred thousands or more. However, three cases have been noted in which over 75 per cent of certain small lots of corms have been found to be affected with Fusarium rot. It is interesting to note that, whereas inoculation experiments indicate the absence of varietal differences

[1] The writer is indebted to the Boyce Thompson Institute for Plant Research for laboratory and greenhouse facilities placed at his disposal during part of the course of this investigation.

in susceptibility, yet in these three cases the disease was more or less restricted to certain varieties.

Although certain workers have given attention to gladiolus diseases, literature contains no reference to this particular disease other than that made by the writer in 1922 (4). It is probable, because of the relatively greater importance of hard rot, dry rot and neck rot or scab (1), that these diseases have received first attention and that one or more of them were involved in cases where the identity of the disease is left uncertain in published accounts.

SYMPTOMS OF THE DISEASE

At the time of harvesting the corms in the autumn, Fusarium rot lesions appear as small water-soaked spots. They are of a reddish-brown color and occur in most cases on the sides and lower halves of the corms (Pl. XXV). It is usually necessary to remove the husks (sheathing leaf bases) from the corms in order to see the lesions, although in some cases the husk is also diseased, as indicated by its discoloration and unusual brittleness.

The rot advances during storage, the rate increasing with higher temperatures and increased humidities. Lesions are usually irregularly circular in outline and range in size from one-fourth to one inch in diameter depending upon conditions of storage and time of year. The entire corm may become involved and reduced to a dry, brownish-black, worthless mummy due to the coalescence of two or more lesions.

Perhaps the most characteristic symptom of this disease is the existence of prominent, irregularly concentric markings or ridges in the lesions (Pl. XXV, C). Unfortunately, the absence of these markings is not absolute proof that the disease is not Fusarium rot; however, they occur more often than not, and the writer has never observed them in lesions produced in any other gladiolus disease. They have been observed consistently on diseased corms of the varieties Fire King, Princeps and Mrs. Francis King.

The lesions are generally somewhat sunken due to the rapid drying and shrinking of the tissues. The advancing margins are usually definite and slightly raised, with active lesions showing a narrow, watersoaked outer area, dark in color, which blends sharply into the healthy tissue. Diseased tissue is hard and in most cases not over 5 to 7 millimeters in thickness. As is true of the hard rot, dry rot and scab, the diseased tissue, especially of less active lesions, can be chipped out with the finger nail, leaving apparently healthy tissue beneath.

The color of the lesions, although generally reddish-brown, varies somewhat with the variety affected. On Mrs. Francis King, hazel[2] is the predominating color; and on Fire King the color range is from burnt sienna to bay, with older lesions almost black.

[2] Designations of colors are according to Ridgway (5).

Fusarium rot is primarily a disease of stored corms, but certain conditions of disease in the field relate directly to the rot which advances during storage. Missing plants in the row may be due to the fact that the corms planted were worthless mummies, their rotted condition being concealed by the apparently healthy husks. Dwarfed, stunted plants, which frequently fail to produce blossoms, result from planting corms so severely diseased that they fail to develop root systems or conducting tissues sufficient to support the developing offspring. Diseased corms commonly decay prematurely in the field, saprophytes, in many if not most cases, playing an important rôle in the rapid disintegration of the tissues.

In two instances the pathogene causing this disease has been isolated from the base of decaying stems, and in one instance from lesions on the roots. In other instances species of *Fusarium* which might have been this fungus have been isolated from stems and roots, but the identities of the fungi were not determined absolutely. It seems certain, however, that the pathogene causing the corm rot may also cause a decay of the stem.

When corms affected with the Fusarium rot are kept under very moist conditions, the lesions commonly become covered with a grayish, mold-like growth of the fungus (Pl. XXV, A). Corms in this condition have been received from growers. Only in relatively few cases, however, has the fungus present on the surface been proved to be the pathogene here described. Under such conditions, common saprophytes are to be expected.

ETIOLOGY

The Fusarium rot of gladiolus is caused by the fungus *Fusarium oxysporum* var. **gladioli** n. var.

SOURCE OF PURE CULTURE

The culture of *Fusarium* referred to in the following descriptions and experiments was isolated from a typically diseased corm of the variety Fire King on February 9, 1925. Tissue plantings were first made, and from a subsequent transfer a strain from a single spore was obtained.

Simultaneously with the studies on which these descriptions are based, cultures of the fungus isolated from other varieties and reisolated from artificially infected corms have been studied sufficiently to eliminate any doubt of the strain from Fire King being pathogenic and being the one most commonly isolated from diseased corms. It is not maintained, however, that other Fusaria may not attack the gladiolus and cause corm rots similar to the one herein described.

MORPHOLOGY

The morphological characters (Figs. 1 and 2) of the organism, as determined from cultures on various media, are as follows:

Microconidia numerous, dominantly 0-septate. Macroconidia gradually attenuated toward both ends, nearly cylindrical in the middle half of their length, somewhat pedicellate, and usually uniformly curved throughout;

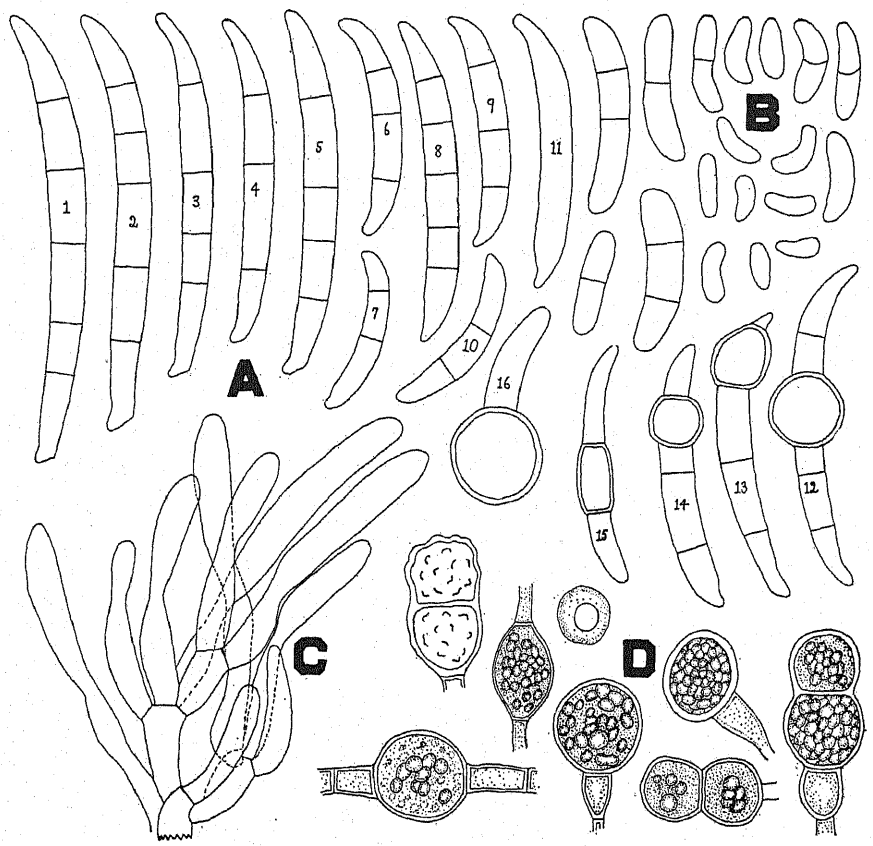


FIG. 1. Spores of *Fusarium oxysporum* var. *gladioli*. A. Macroconidia: 1, 10, from a medium sized sporodochium on hard potato agar after 25 days; 2, from a medium sized sporodochium on potato tuber plug after 39 days; 3, 4, 6, from a small sporodochium on tomato stem after 39 days; 5, 7, 8, from a large sporodochium on corn meal agar after 90 days; 9, 16, from a medium sized sporodochium on cornmeal agar after 38 days; 11, from a small sporodochium on tomato stem after 30 days; 12, 13, 14, 15, from a large sporodochium on tomato stem after 94 days. B, microconidia from culture on hard potato agar after 19 days. C, conidiophore from culture on tomato stem after 8 days. D, chlamydospores from culture on tomato stem after 94 days. Outlined with camera lucida, x 1000.

0-septate up to 98 per cent, 35 x 4.2 (30–42 x 3.7–4.6) μ; 1-septate up to 6 per cent, 20.5 x 3.2 (19–21 x 3.1–3.4) μ; 2-septate up to 10 per cent, 28 x 4.0 (27–30 x 3.9–4.1) μ; 3-septate, dominant, up to 92 per cent, 33.4 x 4.3 (25–41 x 3.5–4.8) μ; 4-septate up to 47 per cent, 39 x 4.4 (33–43 x 3.8–4.8) μ; 5-septate up to 8 per cent, 44 x 4.5 (42–46 x 4.3–4.6) μ; 7-septate, less than 1 per cent, 42 x 4.8 (40–44 x 4.8) μ. Chlamydospores mostly 0-septate, approximately globose and smooth-walled, intercalary or terminal, and commonly present in conidia; in conidia, 0-septate, 9 x 7 (6–14 x 5–10) μ; in mycelium, 0-septate, 11 x 10 (7–17 x 7–16) μ. Aerial mycelium present on most media, typically white and well developed, loosely-cottony, up to 5 mm. in height. Sporodochia up to 2 mm. in diameter, forming in from 7 to 14 days, typically salmon-buff in color. Bluish-black sclerotia present on potato tuber plug and commonly present on other media.

CULTURAL CHARACTERS

Fusarium oxysporum var. *gladioli* was grown on the following media: cornmeal agar, with and without 5 per cent dextrose, in petri dishes, spore dilution cultures; hard potato agar neutral, with and without 5 per cent dextrose, in petri dishes and test tubes; hard potato agar, made acid by the addition of one drop of 50 per cent lactic acid to 10 cubic centimeters of the medium, with and without 5 per cent dextrose, in petri dishes and test tubes; oatmeal agar, in petri dishes and test tubes; lima bean agar, in test tubes; rice, 2 grams in 6 cubic centimeters of water in test tubes; potato tuber cylinders, in test tubes, no water added; sweet clover, raspberry, tomato and potato stems in test tubes, each with 4 cubic centimeters of water added. The vegetable decoctions were prepared according to the formulae given by Sherbakoff (7, p. 105). All agar media contained 3 per cent agar. All media were autoclaved for 45 minutes at 10 pounds pressure, except rice, which was steamed for one hour on three successive days. With the exception of cultures on cornmeal agar, which were spore dilutions, all were initiated by transferring bits of medium containing mycelium from other cultures. The cultures were kept in diffuse daylight at room temperature (approximately 22° C.).

No marked variations or unusual types of growth occurred on any of the media. It was not difficult to maintain the cultures in the state of *Hochkultur* over a period sufficiently long to complete the taxonomic study. The intensity of color production was increased by the addition of lactic acid and of dextrose. The growth rate, as measured by the diameter of the thalli in petri dishes, was slightly retarded by the addition of the lactic acid and slightly increased by the addition of 5 per cent dextrose. Aerial mycelium was present and typically well developed in all cultures with the

exception of those in which the fungus was growing on cornmeal agar. It was 3 to 5 mm. in height, of a loose-cottony appearance becoming slightly more woolly and appressed with age, and dominantly white. In cultures where the aerial mycelium was colored, as for example, hard potato agar with 5 per cent dextrose, the color varied from pinkish vinaceous through dark vinaceous to ox-blood red, representing a diffused color of the stroma. On rice, when 60 days old, the aerial mycelium was dominantly white, ranging to pinkish vinaceous. Sporodochia formation was retarded or entirely prevented by the addition of lactic acid or 5 per cent dextrose. Mature sporodochia were present in cultures on cornmeal agar with 1.5 per cent dextrose in 16 days, and abundant on oat agar in 24 days. They were formed readily and in large numbers on stems of raspberry, tomato, sweet clover, and potato, being mature in about 14 days. They were typically salmon-buff in color.

Only in those cultures in which the fungus was growing in decoctions to which dextrose or lactic acid, or both, had been added, and in cultures on rice and potato tuber plugs, did the substratum become colored. A deep purplish vinaceous color developed in from 5 to 7 days. In about 15 days, in petri dish cultures on potato agar, the color of the substratum became almost black in the center of the dish, grading to the outer edge through bordeaux, dark vinaceous purple and ox-blood red. On rice, when 60 days old, the substratum was dominantly pinkish vinaceous, grading through dark vinaceous to bluish-black deeper in the medium.

In determining the size of the conidia, at least 50 and in some instances 100 spores were measured. The measurements on the various media are as follows:

On cornmeal agar, culture 16 days old; conidia taken from a small sporodochium:
0-septate, 98 per cent, 41.2 x 4.7 (30.0–50.4 x 4.2–6.0) μ.
3-septate, 2 per cent, 42 x 4.8 μ.
On tomato stems, culture 18 days old; conidia taken from a large sporodochium:
0-septate, 92 per cent, 31.5 x 4.6 (25.2–45.6 x 3.6–5.4) μ.
3-septate, 8 per cent, 29.7 x 4.8 (25.2–37.2 x 4.8) μ.
On tomato stems, culture 19 days old; conidia from a large sporodochium:
0-septate, 30 per cent, 35.1 x 4.1 (27.6–49.2 x 3.6–4.8) μ.
2-septate, 4 per cent, 33.6 x 3.6 (31.2–36.0 x 3.6) μ.
3-septate, 52 per cent, 35.1 x 4.0 (27.0–48.0 x 3.0–4.8) μ.
4-septate, 14 per cent, 43.0 x 4.1 (38.4–50.4 x 3.6–4.8) μ.
On potato stem, culture 21 days old; conidia from a medium sized sporodochium:
0-septate, 34 per cent, 29.5 x 3.6 (21.6–36.0 x 3.0–4.2) μ.
1-septate, 2 per cent, 19.2 x 3.0 μ.
2-septate, 10 per cent, 26.2 x 3.7 (19.2–31.2 x 3.6–3.9) μ.
3-septate, 54 per cent, 29.8 x 3.9 (24.0–36.0 x 3.0–4.8) μ.
On potato tuber plug, culture 21 days old; conidia taken from a small sporodochium:
0-septate, 30 per cent, 32.8 x 4.2 (28.8–45.6 x 3.6–4.8) μ.

3 septate, 44 per cent, 30.4 x 4.3 (20.4–38.4 x 3.0–4.8) μ.
4-septate, 24 per cent, 35.0 x 4.5 (30.0–39.6 x 3.6–4.8) μ.
5-septate, 2 per cent, 37.2 x 4.2 μ.

On sweet clover stems, culture 22 days old; conidia taken from a small sporodochium:

0-septate, 7 per cent, 27.8 x 4.0 (20.0–36.0 x 3.3–4.2) μ.
3-septate, 83 per cent, 32.6 x 4.3 (20.0–42.0 x 3.6–4.8) μ.
4-septate, 10 per cent, 37.4 x 4.2 (33.6–40.8 x 3.6–4.8) μ.

On locust stems, culture 22 days old; conidia taken from a small sporodochium:

1-septate, 4 per cent, 24.0 x 3.3 (20.4–27.6 x 3.0–3.6) μ.
2-septate, 2 per cent, 31.2 x 3.6 μ.
3-septate, 86 per cent, 31.3–4.1 (21.6–39.6 x 3.0–4.8) μ.
4-septate, 8 per cent, 34.8 x 4.0 (32.4–37.2 x 3.6–4.5) μ.

On hard oat agar, culture 23 days old; conidia from a large sporodochium:

3-septate, 54 per cent, 34.7 x 4.4 (26.4–46.2 x 3.6–4.8) μ.
4-septate, 44 per cent, 41.5 x 4.4 (34.8–48.0 x 4.2–4.8) μ.
5-septate, 2 per cent, 45.6 x 4.2 μ.

On hard oat agar, culture 25 days old; conidia taken from a medium sized sporodochium:

0-septate, 2 per cent, 39.6 x 3.6 μ.
2-septate, 2 per cent, 32.4 x 4.8 μ.
3-septate, 48 per cent, 35.3 x 4.5 (24.0–45.6 x 3.6–4.8) μ.
4-septate, 40 per cent, 44.2 x 4.5 (36.0–56.4 x 4.2–4.8) μ.
5-septate, 8 per cent, 47.1 x 4.4 (44.4–49.2 x 4.2–4.8) μ.

On hard oat agar, culture 25 days old;

(a) Conidia taken from a medium sized sporodochium:
3-septate, 71 per cent, 34.8 x 4.3 (25.2–44.4 x 3.0–4.8) μ.
4-septate, 28 per cent, 39.1 x 4.5 (33.6–48.0 x 3.6–4.8) μ.
5-septate, 1 per cent, 55.8 x 4.8 μ.

(b) Chlamydospores in mycelium:
0-septate, 100 per cent, 10.6 x 11.0 (6.0–14.4 x 7.2–15.6) μ.

On hard potato agar, culture 36 days old;

(a) Conidia taken from a medium sized sporodochium:
1-septate, 6 per cent, 20.8 x 3.2 (19.2–21.6 x 3.0–3.6) μ.
2-septate, 2 per cent, 25.2 x 3.6 μ.
3-septate, 62 per cent, 33.8 x 4.1 (27.6–39.6 x 3.6–4.8) μ.
4-septate, 30 per cent, 38.4 x 4.2 (36.0–45.6 x 3.9–4.8) μ.

(b) Chlamydospores in conidia:
0-septate, 98 per cent, 8.7 x 6.8 (6.0–10.8 x 4.8–8.4) μ.
1-septate, 2 per cent, 9.6 x 6.0 μ.

On cornmeal agar, culture 36 days old; conidia taken from a large sporodochium:

2-septate, 1 per cent, 19.2–3.6 μ.
3-septate, 55 per cent, 33.2–4.2 (21.6–44.4 x 3.0–4.8) μ.
4-septate, 38 per cent, 38.1 x 4.4 (32.4–48.0 x 3.6–4.8) μ.
5-septate, 6 per cent, 40.8 x 4.7 (38.4–45.6 x 4.2–4.8) μ.

On hard oat agar, culture 38 days old; conidia taken from a large sporodochium:

3-septate, 74 per cent, 32.8 x 4.3 (26.4–39.6 x 3.6–4.8) μ.
4-septate, 18 per cent, 39.6 x 4.1 (33.6–44.4 x 3.6–4.2) μ.
5-septate, 8 per cent, 47.1 x 3.7 (42.0–52.8 x 3.6–4.2) μ.

On cornmeal agar, culture 38 days old;

(a) Conidia taken from a medium sized sporodochium:

2-septate, 2 per cent, 27.6–4.2 μ.
3-septate, 58 per cent, 34.1 x 4.2 (24.0–38.4 x 3.3–4.8) μ.
4-septate, 32 per cent, 38.3 x 4.5 (32.4–48.0 x 3.6–4.8) μ.
5-septate, 8 per cent, 41.1 x 4.7 (38.4–43.2 x 4.5–4.8) μ.

(b) Chlamydospores in conidia:

0-septate, 100 per cent, 9.7 x 8.9 (4.8–15.6 x 4.8–14.4) μ.

On potato tuber plug, culture 39 days old; conidia taken from a medium sized sporodochium:

0-septate, 2 per cent, 38.4 x 4.8 μ.
2-septate, 6 per cent, 21.6 x 3.8 (20.4–22.8 x 3.6–4.2) μ.
3-septate, 78 per cent, 29.3 x 4.3 (20.4–38.4 x 3.6–4.8) μ.
4-septate, 14 per cent, 32.7 x 4.5 (24.0–39.6 x 4.2–4.8) μ.

On tomato stem, culture 39 days old;

(a) Conidia from a small sporodochium:

0-septate, 1 per cent, 38.4 x 4.2 μ.
1-septate, 1 per cent, 15.6 x 3.0 μ.
2-septate, 3 per cent, 32.8 x 4.1 (27.0–38.4 x 3.6–4.5) μ.
3-septate, 83 per cent, 36.8 x 4.4 (19.2–44.4 x 3.6–4.8) μ.
4-septate, 11 per cent, 41.1 x 4.4 (34.8–51.6 x 4.2–4.8) μ.
5-septate, 1 per cent, 40.8 x 4.8 μ.

(b) Chlamydospores in conidia:

0-septate, 100 per cent, 8.3 x 6.6 (6.0–11.4 x 4.8–7.8) μ.

On cornmeal agar, culture 90 days old; conidia taken from a medium sized sporodochium:

1-septate, 1 per cent, 22.8 x 3.6 μ.
2-septate, 2 per cent, 21.0 x 3.9 (19.2–22.8 x 3.6–4.2) μ.
3-septate, 40 per cent, 32.9 x 4.5 (24.0–39.6 x 3.6–5.4) μ.
4-septate, 47 per cent, 36.7 x 4.7 (30.0–50.4 x 4.2–5.4) μ.
5-septate, 8 per cent, 40.8 x 4.7 (34.8–44.4 x 4.2–4.8) μ.
7-septate, 2 per cent, 42.0 x 4.8 (39.6–44.4 x 4.8) μ.

On hard oat agar, culture 92 days old; chlamydospores in conidia:

0-septate, 100 per cent, 9.6 x 6.6 (6.0–16.8 x 4.5–9.6) μ.

On tomato stem, culture 94 days old; chlamydospores in mycelium:

0-septate, 92 per cent, 12.0 x 10.7 (7.8–19.2 x 7.2–15.6) μ.
1-septate, 8 per cent, 19.2 x 12.6 μ.

On locust stems, culture 105 days old; conidia taken from a medium sized sporodochium:

0-septate, 4 per cent, 33.6 x 4.1 (30.0–37.2 x 3.6–4.5) μ.
3-septate, 92 per cent, 32.3 x 3.8 (21.5–44.5 x 3.0–4.8) μ.
4-septate, 4 per cent, 41.2 x 4.2 μ.

Average of the above measurements:[3]

Conidia: 0-septate, 30 per cent, 34.8 x 4.2 (30.0–41.6 x 3.7–4.6) μ.
1-septate, 3 per cent, 20.5 x 3.2 (19.4–21.4 x 3.1–3.4) μ.
2-septate, 3 per cent, 28.4 x 4.0 (26.8–29.9 x 3.9–4.1) μ.
3-septate, 58 per cent, 33.4 x 4.3 (25.0–41.0 x 3.5–4.8) μ.
4-septate, 26 per cent, 38.6 x 4.4 (33.0–42.7 x 3.8–4.8) μ.

[3] Percentages calculated on the basis of cultures in which the indicated conidia were found—not on total number of cultures studied.

5-septate, 5 per cent, 44.0 x 4.5 (41.9–46.1 x 4.3–4.6) μ.
7-septate, less than 1 per cent, 42.0 x 4.8 (39.6–44.4 x 4.8) μ.

Chlamydospores:

(a) in conidia, 0-septate, 99 per cent, 9.1 x 7.2 (5.7–13.6 x 4.7–10.5) μ.
(b) in mycelium, 0-septate, 96 per cent, 11.3 x 10.9 (6.9–16.8 x 7.2–15.6) μ.

From a comparison with descriptions by Wollenweber (9), Sherbakoff (7) and Wollenweber *et al.* (10), it is evident that this fungus (Figs. 1 and 2) falls in the section Elegans of the genus *Fusarium,* and within that group lies so close to *Fusarium oxysporum* Schlecht. emend. Wr. (6, 8) as to be considered a variety of this well-known species. It differs from *F. oxysporum* in having slightly longer macroconidia, a higher percentage of 4-septate spores, somewhat weaker development of the "foot" (Figs. 1 and 2), and especially in its parasitism (see under pathogenicity, below). For these reasons it would seem that the gladiolus fungus should be made at least a new variety, the alternative being to make it a new species.[4]

PATHOGENICITY

The ability of *Fusarium oxysporum* var. *gladioli* to infect the gladiolus and produce lesions on corms typical of those from which the fungus has been isolated many times has been established through numerous experiments both in the field and under greenhouse conditions. Infection takes place when the fungus is added to the soil before the corms are planted, when the corms are dipped in suspensions of spores from a pure culture and either planted in soil or else held in moist chambers in the laboratory, and when inoculations are made with the pathogene into newly developing corms from healthy corms planted in clean soil.

On March 28, 1916, 12 plants, variety Meadowvale, growing in the greenhouse bench were inoculated when they were from 8 to 10 inches in height. The soil was removed from about the corms and a bit of potato agar carrying the fungus was pressed against each of the uninjured corms. The inoculum was then covered with cotton saturated with water, and the soil replaced. When an examination was made on April 12, at which time the experiment was discontinued, it was found that lesions typical of Fusarium rot were present on 8 of the 12 corms. Check plants remained healthy. The fungus was reisolated from these lesions and its identity determined.

In the summer of 1921 infection was obtained by inoculating corms of the varieties Princeps, Augusta, Peace, Mrs. Francis King, America, Fire King, Meadowvale, and three unknown varieties, as well as Primulinus

[4] Assistance in the determination of the fungus was given by Dr. C. D. Sherbakoff, who concurred with the writer in his decision that the fungus should be made a variety of *F. oxysporum* Schlecht. emend. Wr.

Hybrids. These were growing in an experimental garden in soil in which no gladioli had ever been grown. The parent corms were planted June 1, and the corms developing from them were inoculated during August when they were approximately one inch in diameter. Of the 15 corms of each

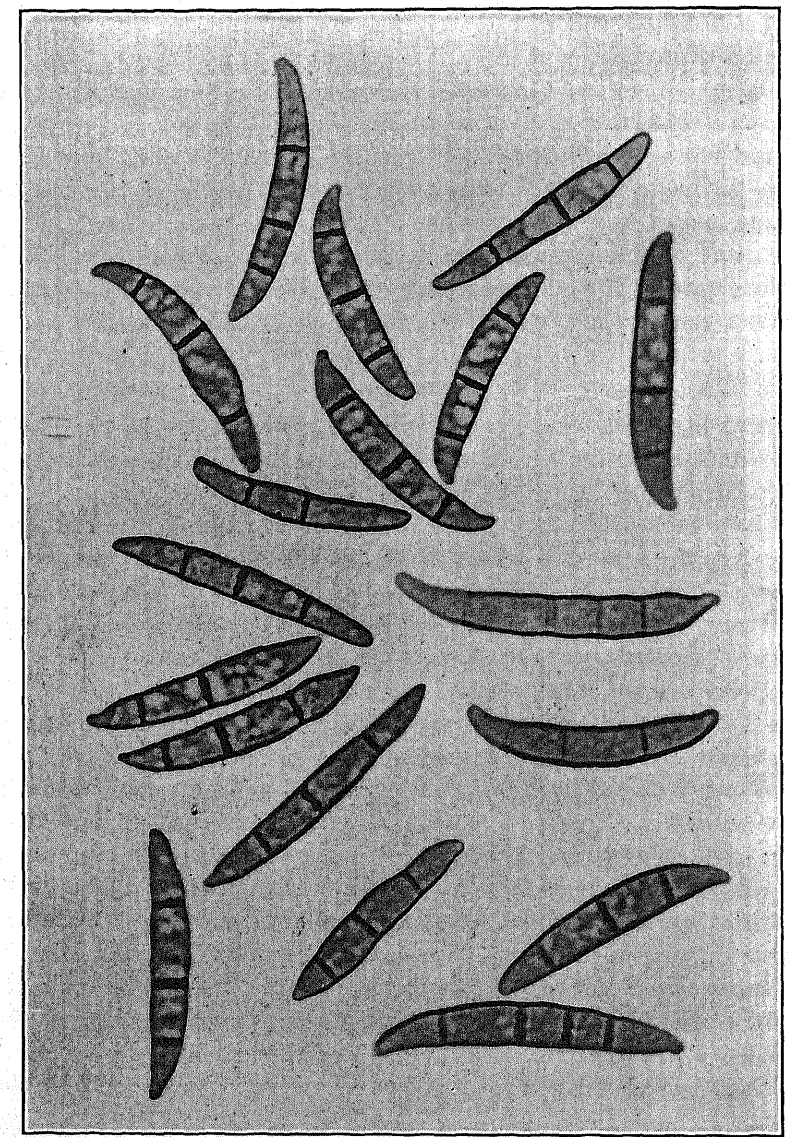


FIG. 2. Macroconidia of *Fusarium oxysporum* var. *gladioli*, from medium sized sporodochium on sweet clover stem after 63 days. Photomicrograph, x 800.

variety inoculated, 5 were injured by making several cuts through the sheathing leaf bases into the body of the corms, and the remainder uninjured. The inoculum was taken from a two months' old culture of the fungus on sterilized wheat kernels. The method involved pulling away the soil from the base of the plant and pressing about six kernels of wheat carrying the fungus against the injured or uninjured surface. The inoculum was then covered with cotton saturated with water and the soil replaced. Five plants of each lot were given similar treatment with the exception that the fungus was omitted. No evidences of disease, as indicated on plant parts above ground, developed prior to harvest in October, and the corms were not examined until the following January. Infection was obtained on all varieties, and the checks were found to be sound. The percentages of infection were 90 and 68, respectively, of those injured and uninjured.

That infection may be obtained under artificial conditions was proved by the following experiment. Fifty large corms of the variety Mrs. Francis King were selected as being sound, no lesions being evident from a critical examination following the removal of the husks. They were then placed in 50 per cent alcohol for 3 minutes, in 1–1,000 mercuric chloride for 15 minutes, given five washings in distilled water, and then placed on moistened filter paper in moist chambers, 240 by 80 mm., which had been sterilized previously. Two kinds of inoculum were used. The first consisted of bits of medium bearing the fungus, taken from a 9-days old culture on potato agar; the second, a suspension of spores from sporodochia produced on tomato stems. In the first case the method consisted in pressing the inoculum against the uninjured surface of the corms. In using the second type of inoculum, somewhat less than one cubic centimeter of the spore suspension was dropped into the depressions occurring naturally on the upper surfaces of the corms. Each inoculated lot consisted of 20 corms, and 10 corms were carried as checks. The moist chambers were kept in a constant temperature chamber at 25° C., and water was added as needed to keep the filter papers saturated.

The inoculations were made on April 30, 1921. On June 10, lesions measuring from one-half to one inch in diameter were present on all the inoculated corms. By July 15, the rot involved from one-half to the entire surface of the corms, and a mold-like growth of the fungus covered the lesions. This growth was powdery in appearance, due to the large numbers of spores, and numerous sporodochia also were present. The checks remained free from decay.

Several other experiments similar to the above have been conducted at various times and have always yielded a high percentage of infection. Some of the earlier experiments gave results indicating that varieties with dark

colored corms were more resistant than those with white and light yellow corms, but later experiments failed to substantiate this possibility.

Infection has been secured by inoculating uninjured stems at a distance of 8 to 12 centimeters above the surface of the soil. In all cases the inoculum was covered with cotton, which was kept moist by adding water from time to time during the first week following inoculation. In about three weeks the rot had advanced down the stem to below the soil line and had progressed above the point of inoculation about 5 centimeters. The foliage became yellow and died prematurely.

No infection with *F. oxysporum* var. *gladioli* has been obtained in several trials on living stems of tomato, pepper, egg-plant and potato. In one experiment, negative results were obtained on potato plants under conditions which yielded infection with a culture of *F. oxysporum* obtained from Dr. C. D. Sherbakoff. The inoculations were made by introducing the fungi growing on wheat kernels into longitudinal slits in potato stems at about the surface of the soil. The plants were growing in the greenhouse and were from 6 to 8 inches in height when inoculated. The stems at the point of inoculation were wrapped with moistened cotton which was not removed throughout the duration of the experiment. Twenty stems were inoculated with each of the two fungi. The check consisted of the same number of stems given similar treatment, except that no fungous growth was introduced into the stems along with the wheat kernels.

The inoculations were made on August 15, 1925. On September 15 it was found that 15 of the 20 stems inoculated with *F. oxysporum* were infected, as evidenced by the discoloration of the vascular tissues for a distance of about 2.5 centimeters below and 6.5 centimeters above the point of inoculation. No pronounced wilting was observed. Tissue plantings were made from these stems and the fungus obtained in culture. In the case of the gladiolus fungus, a very slight discoloration, less than 1.5 centimeter in total extent, occurred at the point of inoculation, but there were no indications that the fungus was pathogenic to potatoes. It would seem, then, that the parasitism of *Fusarium oxysporum* var. *gladioli* is not identical with that of *Fusarium oxysporum* from potatoes.

TEMPERATURE RELATIONS

Cultures of *F. oxysporum* var. *gladioli* were established by transferring bits of a solid medium carrying mycelium to the center of petri dishes of equal size containing equivalent amounts of hard potato agar with 1 per cent dextrose. The medium was tested electrometrically and found to have a pH value of 7. After incubating all of the cultures for 24 hours at 20° C., they were divided into lots of four dishes each and each lot held for 10

hours at the particular temperature at which it was to be kept throughout the experiment. This permitted the fungus to establish itself in the medium in the petri dish and eliminated errors that might have arisen from failure to allow sufficient time for temperature adjustments before making the initial measurements. The diameters of the thalli were then recorded and the rate and amount of subsequent growth determined by measuring the increases over the original diameters. Four cultures were placed in each of a series of temperature chambers with the following temperatures: 0°, 5°,

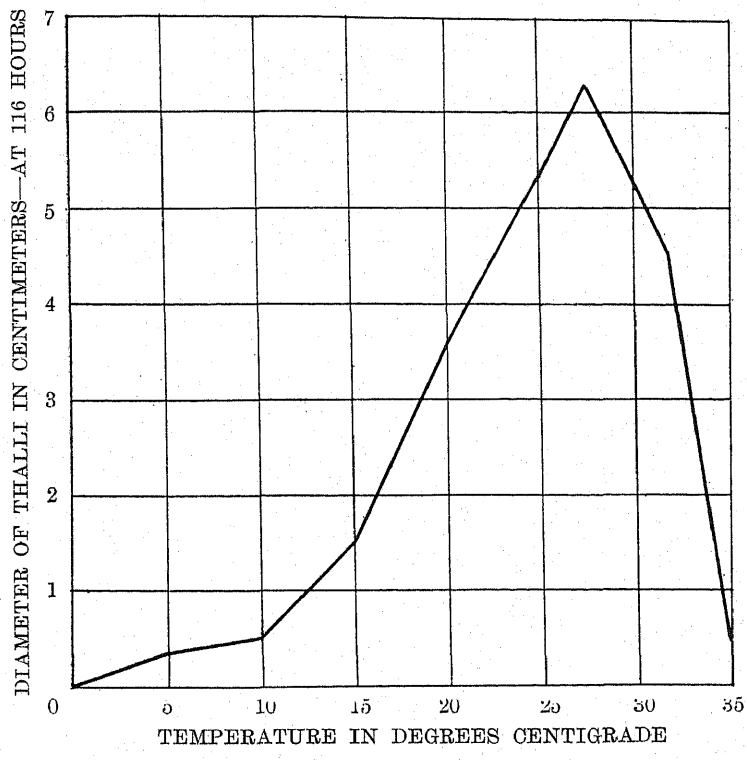


FIG. 3. Relation of temperature to growth of *Fusarium oxysporum* var. *gladioli* on hard potato agar with 1 per cent dextrose in petri dishes.

10°, 15°, 20°, 25°, 27.5°, 30°, 32° and 35° C. The diameters of the thalli at 116 hours were taken as a criterion of comparison, the results being represented graphically in figure 3. There is a gradual increase in rate of growth up to 25° with optimum near 27.5°, and a rapid drop beyond 30° C.

CONTROL

No experiments in the control of this particular disease have been attempted. From what is known regarding the nature of the disease, its

cause and the life history of the pathogene, it would seem that the problem of control would not differ greatly from that of the hard rot and dry rot diseases. For the latter two, it has been found that sorting, with the elimination of diseased corms, combined with a four or five years' rotation and proper handling of the corms at harvest and during storage are usually sufficient to keep the losses at a minimum. To be properly handled, the corms should be dug when they can be dried in the open for a day or two before being placed in storage. As little soil as possible, either adhering to or accompanying the corms, should be taken indoors. The old roots and adhering soil should be removed early during the storage period.

Throughout the first month the storage house should be given maximum ventilation to carry off the moisture and to facilitate drying. As early as possible in sections with cold winters, as in New York State, the temperature should be dropped to about 35° F., where it should be held throughout the winter.

Besides maintaining a low temperature, any practices which lower the humidity of the air about the corms is to be recommended. In instances where their use is feasible, trays with slatted rather than solid bottoms are preferred, and these are best stacked in racks which will permit the movement of air on all sides of them. The corms should not be piled deeper than 4 inches in the trays, and where possible should be stirred several times during the winter. Sorting, with the elimination of diseased corms, should be done late in the spring, since at this time the advanced stage of decay makes it easier to pick out those that are affected.

SUMMARY

Fusarium rot is an important disease of gladiolus corms in the United States. The disease probably occurs to some extent in all collections of gladioli of any considerable size. A description of the disease is given. Corms become infected in the field, and the rot advances during storage. Diseased corms in storage may be reduced to worthless mummies under favorable conditions for the disease, and the planting of infected corms frequently results in the death of plants in the field during mid-season.

The pathogene, *Fusarium oxysporum* var. *gladioli* n. var., belongs to the section Elegans, differing from *F. oxysporum* Schlecht. emend. Wr. in certain morphological characters and in its parasitism. Its morphological and cultural characters are recorded.

On potato agar, the fungus grows over a range of from about 5° to 35° C. Optimum growth takes place at about 27.5° C.

Infection was readily obtained on uninjured corms in the laboratory and on those growing in the greenhouse and experimental gardens.

Although no experiments on control were attempted, it is believed that sorting, with the elimination of diseased corms, together with crop rotation and the proper handling of corms at harvest and during storage, will greatly reduce losses from the disease.

CORNELL UNIVERSITY,
ITHACA, N. Y.

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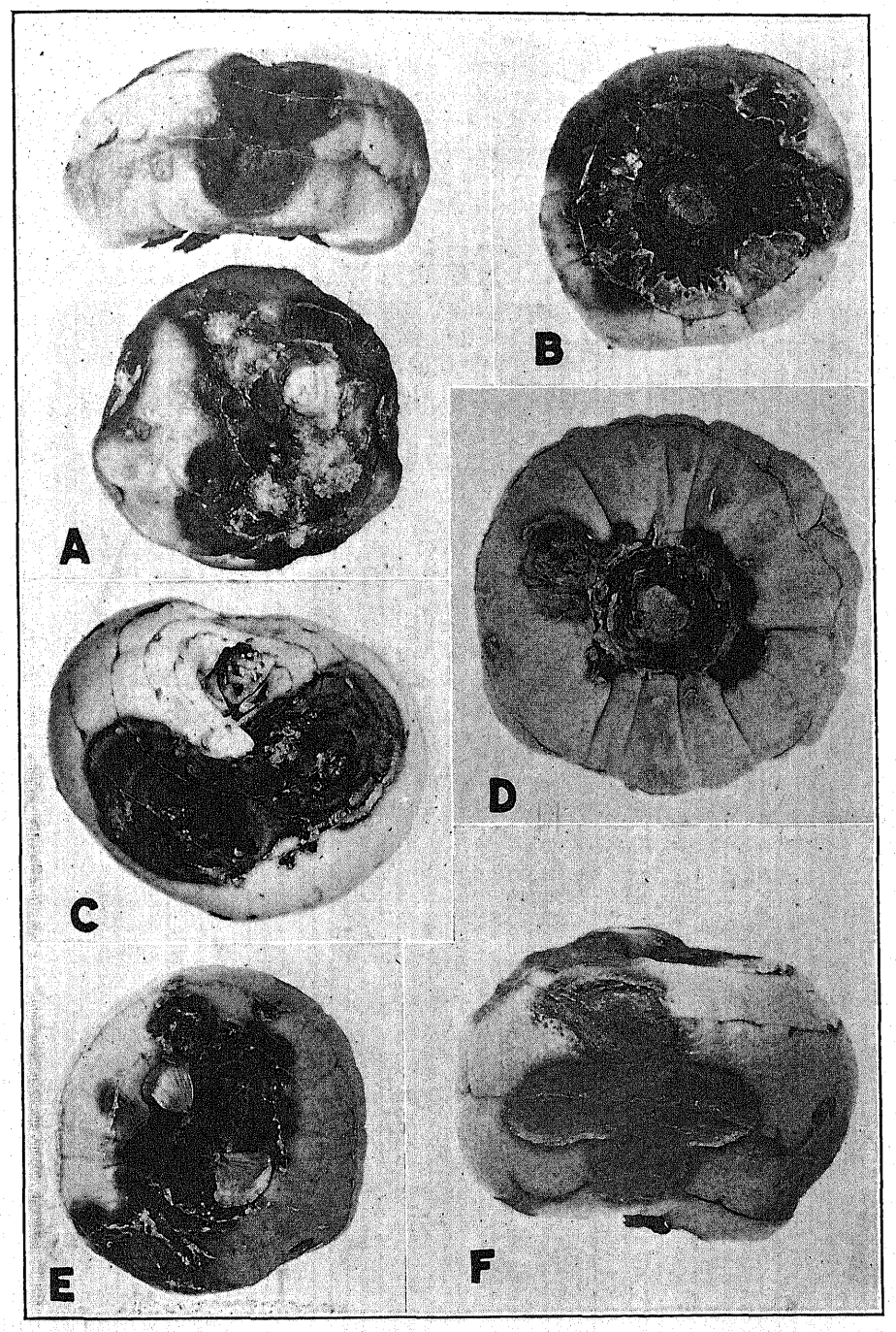
EXPLANATION OF PLATE XXV

Corms affected with *Fusarium oxysporum* var. *gladioli*. A. Variety Princeps, natural infection. B, E. Variety Fire King, natural infection. C. Variety Mrs. Francis King inoculated with pure culture of fungus isolated from Fire King and kept in moist chamber at 24° C. Photographed after 30 days. D. Variety America, natural infection. F. Unknown variety inoculated with pure culture of fungus isolated from Princeps and kept in moist chamber at 24° C. Photographed after 28 days.

All natural size with the exception of F which is x $1\frac{2}{3}$.


A
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SOME FACTORS AFFECTING THE PATHOGENICITY OF ACTINOMYCES SCABIES[1]

G. B. SANFORD[2]

INTRODUCTION

Common scab of potato, *Solanum tuberosum,* caused by *Actinomyces scabies* (Thaxter) Güssow, is prevalent and destructive. All attempts effectively to control the disease have failed. The potato scab pathogene is normally a soil saprophyte, and its activities are therefore influenced by other soil microorganisms. There may be metabiotic or antibiotic relations between these organisms, and these relations, as well as the soil flora, may be altered when one or more of the physical or chemical factors of the soil are changed, but the degree of this modification and its effect on scab development is not well understood.

A review of the work done for the control of potato scab previous to 1914 has been given by Lutman and Cunningham (9). Drechsler (3) has studied the morphology of *Actinomyces,* and Waksman (17) the physiology. Others have studied various methods of reducing scab. In addition to tuber disinfection, the principal preventive measure used has been to attempt to increase the acidity of the soil. This appears to have serious economic and other limitations on many potato soils, especially those having a high initial pH value and which are highly buffered. The plowing down of green crops, particularly rye, often is recommended as a control measure for potato scab, as the decomposition of organic matter thus put into the soil is assumed to increase soil acidity.

OBJECTS OF THE INVESTIGATION

The objects of this investigation were: to study the effect of temperature, moisture, oxygen supply, soil reaction, and stimulatory substances on the germination, growth and pathogenicity of *A. scabies*; and to throw additional light on the nature of the control said to be obtained by plowing under a green crop of rye.

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[2] Grateful acknowledgment is made to Professor E. C. Stakman for his assistance and helpful criticism during this study.

METHODS

A synthetic dextrose-nitrate substrate was used for all studies, except where otherwise stated, the composition being: K_2HPO_4, 1 gm; $MgSO_4$, 0.5 gm; KCl, 0.5 gm; $FeSO_4$, trace; $NaNO_3$, 2 gm; dextrose 30 gm; agar 18 gm; distilled water 1000 cc. Agar slants in loosely plugged test tubes were found preferable to petri dish cultures. It was found advisable to add the spores[3] in suspension to the surface of the solid medium. Other methods will be described when necessary. A strain of *A. scabies* of tested pathogenicity was labelled "B" and used in all studies, except where otherwise stated.

EXPERIMENTAL RESULTS

Temperature Relations

The effect of temperature on spore germination has received attention by Shapovalov (15) and others, but a study of the influence of temperature on the pathogenicity of the strain of *A. scabies* used in these experiments seemed advisable.

Effect on growth and pathogenicity. Three strains of *A. scabies* (A, B and C) were used. Strains B and C were obtanied from S. A. Waksman, Rutgers College, N. J., and strain A was isolated by the writer and tested for pathogenicity. Duplicate cultures on nutrient agar slants were kept at the following temperatures: 7–8°, 10°, 13°, 16°, 19°, 22°, 24°, 26–27°, 29–30°, 32°, 35°, 37–38° C., and the growth recorded at 18-, 36-, 60-, 80- and 160-hour intervals. This experiment was repeated three times. All three strains reacted alike. The data for strain B are given in table 1.

TABLE 1.—*The effect of temperature on the growth of Actinomyces scabies, strain B*

Time in hours	Temperature in degrees Centigrade											
	7–8	10	13	16	19	22	24	26–27	29–30	32	35	37–38
	Rate of growth[a]											
18	0	0	0	0	0	1	2	4	5	4	2	0
36	0	0	0	0	1	3	4	4	6	5	3	0
60	0	0	0.5	1	4	5	6	7	8	6	3	1
80	0	trace	2	3	6	7	10[b]	12[c]	10	6	2	2
160	0	5	8	9	10	11	12[c]	12[c]	10	5	2	1

[a] Estimated value given to the relative progress of cultures after germination.
[b] Darkening.
[c] Dark.

[3] "Spores" is used in the widest sense because both conidia and mycelial segments function as reproductive structures.

It is evident from results that the range of temperature for growth increases with the time, and that germination and maturity take place sooner at the higher temperatures, with a corresponding decrease in vegetative growth. For instance, after 18 hours growth occurs between 22° and 35° C., while after 160 hours the range of temperature for growth extends from 10° to 38° C. Although good growth and maturity are attained at the lower temperatures in a longer time, the optimum point for greatest vegetative development and earliest maturity is 26–27° C. These results agree reasonably well with those of Lutman and Cunningham (9) who reported the optimum temperature to be 25° C.

Jones, McKinney and Fellows (6) stated that scab developed best at about 22° C., and that the amount decreased toward 11° and 30.5° C., where scab became negligible, as did also the yield of the tubers. They also suggested that the immediate effect of high temperature is more closely correlated with tuber development than with the growth of the pathogene. Scant growth of individual tubers, however, would not always explain the decrease of scab, because large scab pustules frequently form both on small and large tubers grown under field conditions. High temperatures may retard or prevent the growth of the scab pustule, subsequent to infection, but our knowledge of the process of tuber infection and scab development is insufficient to permit of conclusions.

The writer (14) obtained a maximum amount of scab in soils held at constant moisture content and temperatures of 14°, 17°, and 25° C.; and there was every indication that much scab would develop at at least two degrees lower or higher than 14° and 23° respectively. These results, with those of the present study, indicate that the growth of the pathogene in pure cultures, within the range of temperatures tested, is fairly well correlated with the amount of scab which develops. It might be concluded that temperatures lower than 12° C. will largely prevent scab development under field conditions and that periodic fluctuations between this point and 25° C. would not greatly affect the amount of scab. However, with various biologic and other factors, and a critical period for infection, temperature may prove to be more important in the field than it appeared to be under the controlled soil conditions tested.

Effect on viability. To determine the thermal death point of *A. scabies,* spores from a mature culture were soaked in water for thirty minutes and a very small portion of the spore suspension was put into 5 cc. of sterile water at the temperature under test. At ten- and twenty-minute intervals, portions were withdrawn and spread on cold agar plates to incubate. Repeated tests showed that spores germinated after having been exposed for twenty minutes at 83° but not after exposure at 85° C. They failed to germinate when exposed for ten minutes at temperatures higher than 90° C.

Since the spores of the scab organism resisted such high temperatures, it was decided to test the efficacy of the hot formaldehyde treatment. Experiment showed that scab spores on tubers were killed when immersed for two minutes in the hot formaldehyde solution (2 pints to 30 gallons of water) at 53° C. It also seemed desirable to test the efficacy of the acidulated mercuric chloride treatment for killing spores, because, if successful, its use would obviate the difficulty of the heating required with the formaldehyde treatment. The formula found effective by Cunningham (1) for killing sclerotia of *Rhizoctonia solani* was used. This method consists of soaking the tuber in a 1–1000 solution of mercuric chloride in water, to which has been added 5 cc. of commercial hydrochloric acid per liter. The tubers are kept moist for 24 hours after soaking. Experiments by the writer showed that this method was equally effective in killing spores of *A. scabies* and Rhizoctonia sclerotia.

Oxygen Requirements

Experiments were made to obtain more information on the relation of oxygen to the germination of the spores, the vegetative growth and fructification of *A. scabies,* because the pathogenicity of this soil fungus and the development of scab may be modified by the available oxygen in the soil.

Effect on germination and growth. A simple preliminary experiment suggested the strongly aerobic qualities of *A. scabies.* Six agar slants were inoculated with a spore suspension of the organism. Three tubes were sealed, and three were loosely plugged with cotton. The very faint growth which appeared after 30 hours in the sealed tubes was a striking contrast to the abundant development in those which were loosely plugged. No further increase occurred in the sealed tubes during 30 days. Apparently the available supply of oxygen had been exhausted during the first 30 hours. Another experiment, using the Büchner tube apparatus with alkalinated pyrogallic acid, demonstrated that the spores of *A. scabies* do not germinate in the absence of oxygen.

The relation of pure oxygen and nitrogen gas to the germination of the spores of the scab organism was also tested. These gases were allowed to displace the air in culture flasks, and then the flasks were sealed. No spores had germinated in 30 days in either gas, but, when exposed again to air, those which had been in pure nitrogen germinated and grew well. The spores which had been in oxygen were dead.

The effect of very limited and measurable amounts of oxygen on spore germination was also studied. Partial pressures, ranging from 0 to 29 inches, were obtained in test tubes containing agar slants which had been evenly inoculated with a spore suspension. Fresh inoculum was spread on the surface of the slants at the time the experiment was begun. The tubes

were fitted with rubber stoppers and glass tubes, and then sealed by heat after the air had been exhausted. By repeated experiment the results shown in table 2 were obtained. At zero partial pressure the spores required all the oxygen present in a 25 cc.-test tube to germinate and produce a very scant growth, while at a partial pressure of about 29 inches there was no visible sign of germination when examined microscopically. At a partial pressure of 28 inches short germ tubes appeared on some spores, but most of them did not germinate. The growth was very meager at a partial pressure of 27 inches. At partial pressures 25, 20, 15, 10, and 5 inches, a gradual increase in surface growth was evident over that obtained at a partial pressure of 27 inches, and increasingly higher percentages of spores also germinated at the lower partial pressures. The growth in the control tubes, which were loosely plugged with cotton, was in striking contrast to that at a partial pressure of 0 inches.

Effect on maturity. The relation of a limited amount of oxygen to vegetative growth, fructification, and especially to maturity (the darkening process of aerial hyphae) of *A. scabies* was investigated. Three inoculated petri dish cultures were placed in a large desiccator, a quantity of alkalinated pyrogallic acid calculated to absorb nearly all the oxygen was put at the base, and the cover sealed. White vegetative growth became evident in the control cultures in 24 hours and continued to increase, and on the fourth day the hyphae darkened rapidly. The test cultures grew as well as the controls up to the third day, but they failed to darken or to develop further during eleven days. When air was admitted, the retarded cultures grew quickly. In twenty hours they had darkened very noticeably, and by forty-eight hours were almost as dark as the controls. No detached spores and segments of mycelium were evident when the retarded cultures were examined on the eleventh day, but a later examination of the mature cultures revealed both the spores and segments common to ripe hyphae.

Relation of quantity of oxygen to partial pressure. While observing the germination and growth of *A. scabies* at the high partial pressures, it was suggested that the fungus might not be able to obtain the small quantity of oxygen left in the test tube; that is, the quantity of oxygen and not the partial pressure becomes the limiting factor to growth. No data on this point were found in the literature. Accordingly, a partial pressure of about 29 inches was obtained in two 25 cc. test tubes containing inoculated agar slants, and also in two 11,550 cc. bottles each containing one inoculated agar slant. The partial pressure was tested both in the large and small containers after ten days and found to have remained at about 29 inches. At this time, meager growth, similar to what had occurred in the test tubes at a partial pressure of 10–5 inches (see table 2) could be seen on the slant contained in each of the large bottles. The spores in the small test tubes at

the same partial pressure failed to germinate. The experiment was repeated three times with consistent results. These results suggest that this fungus is able to obtain practically all the oxygen left in a large volume, regardless of partial pressure. Therefore, it appears that quantity of oxygen, and not partial pressure, is the limiting factor to germination and subsequent development of *A. scabies*.

These studies show that *A. scabies* is very dependent on an abundant oxygen supply for quick germination and rapid vegetative development, and also for the ripening process, which means the darkening of the aerial

TABLE 2.—*The effect of various quantities*[a] *of oxygen on spore germination and growth of Actinomyces scabies*

Partial pressure in inches	Growth[b] and germination of spores after				Remarks
	24 hrs.	48 hrs.	72 hrs.	15 days	
29	0	0	0	0	No germination
28	0	0	0	0	Short germ tubes on a few spores
27	1	1	1	1	Extremely meager growth
25	2	2	2	2	More spores germinated; meager growth
20	2	2	2	2	*do*
15	2	2	2	2	Better germination
10	3	3	3	3	Faint growth
5	3	3.5	4	4	Faint growth
0	5	6	6	6	Better growth
Control[c]	25	75	95	100	Excellent ashen colored growth after 72 hours

[a] Obtained by partly exhausting the air from a 25 cc. space in test tubes; barometer reading 72.6 cm.

[b] Estimated relative value of growth at 26° C.

[c] Cultured in loosely plugged test tubes.

hyphae accompanied by easy disintegration of hyphal segments and spores for inoculum. No definite data have been secured relative to oxygen requirements in the soil, or the oxygen-supplying ability of soils of various types and moisture contents; but it seems quite probable that lack of sufficient oxygen is often an important factor in limiting sporulation, spore germination and vegetative development of the pathogene in the soil. This would then affect the ability of the parasite to cause infection and may help to explain why scab is not so prevalent in wet soils as in drier ones.

Moisture Relationships

From 1920 to 1923 the writer (13, 14) found that very little scab occurred when the soil was kept constantly very moist, but abundant scab

appeared in the same soil kept very dry. It was shown that the control obtained in the moist soil was not due to increased acidity, because the dry soil proved slightly more acid than when wet. It was thought that the pathogene might not sporulate as well in saturated soil air, or, possibly, the germination and growth might be delayed by the excess soil moisture around the spores.

Growth on solid media. Equal portions of spore suspension were spread on nutrient agar in petri dishes and cultured in various relative humidities, ranging from 1 to about 95 per cent. Saturated solutions of the following chemicals were used to obtain the relative humidities indicated: H_2SO_4, less than 1 per cent; LiCl, 7.16 per cent; $CaCl_2$, 25.94 per cent; NaOH,

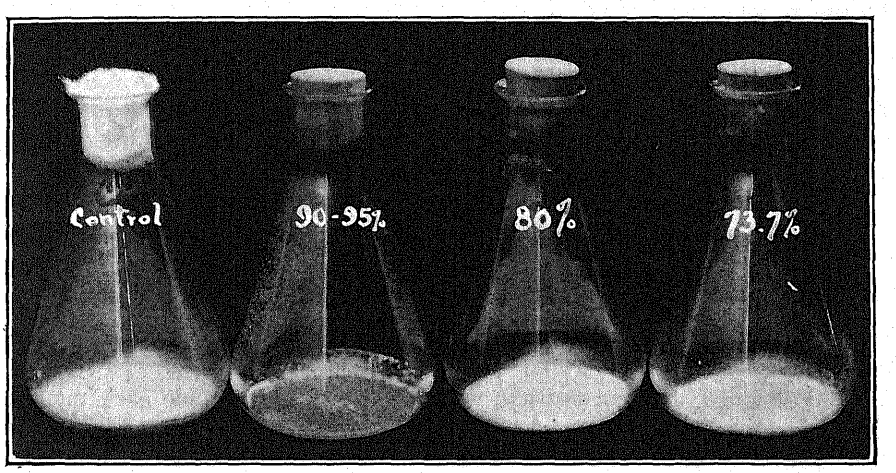

FIG. 1. Growth of *A. scabies* at various relative humidities. Note delayed germination and growth in second flask.

30.72 per cent; $AlCl_3$, 37.1 per cent; $Cu(NO_3)_2$, 45.7 per cent; NaCl, 73.7 per cent; $NaNO_3$, 80 per cent; K_2SO_4, 89.7 per cent; distilled water 90 to about 95 per cent. Owing to the surface condition of the agar when dried too quickly, no satisfactory data were obtained by this preliminary trial, although it was evident that excess water on the substrate delayed growth. In another experiment, saturated air was led successively through distilled water, and a saturated solution of sodium nitrate and sodium chloride. Between each of these solutions, and connected with them, was a 200 cc. flask containing 10 cc. of nutrient agar and 0.5 cc. of spore suspension over the surface of the medium. Within 15 hours vigorous growth appeared in the flask whose humidity was controlled by sodium nitrate, but not until after 60 hours in the flask whose humidity was determined by distilled water (figure 1). Final inspection showed that the cultures retarded by excess

moisture still lagged in development behind those getting an optimum start. This experiment was repeated a number of times. It is suspected that the film of water around the spores remained longer at the higher humidity than at the lower ones, possibly limiting the oxygen supply. It was repeatedly observed that dry spores put into water tend to remain on top, but, if the liquid is shaken frequently and the spores kept immersed, germination may be greatly delayed. If left, however, the spores on top germinate, while those immersed usually remain dormant. It was found that optimum conditions, good vegetative growth can be secured in 24 hours, spore suspension evenly over the surface of agar slants in loosely plugged test tubes, and incubating them in an upright position at 27° C. Under optimum conditions, good vegteative growth can be secured in 24 hours, and a fair degree of maturity (as indicated by the dark color of the aerial growth) in 48 to 60 hours, but this may be greatly postponed by less favorable conditions, even at optimum temperature.

Viability of spores. Studies were made to determine the effect of relative humidities at various temperatures on the viability of the spores of *A. scabies.* A small amount of the aerial hyphae from mature cultures was placed in small sterile phials. These were lightly plugged and placed in sealed jars. Relative humidities of approximately 95, 80, 46, 37, 7 and 1 per cent were obtained by using distilled water, saturated solutions of sodium nitrate, copper nitrate, aluminium chloride, lithium chloride, and concentrated sulphuric acid, respectively. A series of each was incubated at 30°, 26° and 12° C., respectively. Viability tests were made each month. After six months, the spores germinated well, regardless of temperature or humidity.

Soil moisture and increase of inoculum. The amount of inoculum in the soil during the susceptible period of the tuber is an important factor influencing the amount of infection. The soil dilution and plate method was used to determine the relative effect of moist and dry soils on the increase of inoculum. Eight grams of finely-sifted, oven-dry soil was put into a 200 cc. flask and sterilized with dry heat. Five cc. of spore suspension was put into each flask, and after the moisture in the inoculum had diffused, the soil was thoroughly mixed, then various additional amounts of sterile water were pipetted into the flasks and distributed as evenly as possible. The culture flasks were in triplicate in each series. Provision was made for aeration, and the soil cultures set to incubate in a high humidity at 26° C. for 30 days. Dilutions were made by adding enough sterile water to the flasks so that each would finally contain 100 cc. The contents of each flask were then emptied into 900 cc. of sterile water. After shaking and allowing the contents to settle for 30 seconds, a 100 cc. portion was withdrawn from a uniform depth and introduced into another 900 cc. of water. Further

dilutions of 10–90 cc. were made, and a 1 cc. portion of the final dilution was pipetted into each of four culture plates. This method of dilution was used to reduce the error arising from the possible unequal distribution of the inoculum added to the culture flasks, and the very high probable error that was found by repeated trials to exist when only 1 cc. portions were withdrawn from the first dilution. Even this method leaves much to be desired, as the probable error is still high.

The results shown in table 3 indicate that the inoculum failed to increase in the driest soil (6.25 per cent), but at 10, 15, 21.2 and 27 per cent soil moistures it continued to increase until it reached about five-fold its original amount. A sudden decrease is observed at 33.7 per cent soil moisture. Possibly this is on account of the lack of fructification, caused by the hyphae in the very moist soil retaining their vegetative state and failing to break up into segments. The increase at 37 per cent soil moisture is abnormal since in this excessively wet soil the spores were carried by free water to the surface, where they germinated, and where sporulation later occurred.

TABLE 3.—*The effect of soil moisture on increasing the inoculum of Actinomyces scabies cultured in a sandy loam*

Soil moisture in per cent.....	6.25	10	15	21.2	27	33.7	37
Average no. colonies obtained from diluting 80 grams soil	7.1	20.2	31	38	34	17	45[a]
Per cent increase over control (7.1)		184.5	336.6	435.2	378.8	139.4	533.8

[a] This abnormal increase is the result of surface growth on the excessively wet soil.

Soil moisture and scab development. According to the results of the previous experiment and the behavior of the pathogene on nutrient agar, favorable conditions for production of abundant inoculum, and for subsequent growth, would not be found either in excessively dry or in very moist soil but in one of intermediate moisture content. However, the writer (13, 14) found that a maximum amount of scab may develop in a very dry soil.

In one instance tubers developed to maturity in soils having about 4 per cent of moisture by weight (oven-dry basis). The water was led by a tube below the surface soil, in which the tubers formed, to the roots of the plant. It was thought sufficient moisture transpired from the tuber in so dry a soil to enable the spores adhering closely to the surface, or about the lenticels, to germinate, and that the germ tubes grew toward the source of

moisture. Perhaps increased infection of the tuber in dry soils may be due to a hydrotropic response, or the lenticel structure and hydrotropism both may affect it. When once established on the host, the pathogene would develop regardless of the lack of soil moisture, and a dry soil would allow good aeration. Lutman (8) states that scab may originate at any place on the surface of the tuber, and that it most frequently occurs at the lenticels. From extensive observation, the writer believes that the lenticels are the principal infection courts, and are the centers from which the typical scab pustules develop. It is common knowledge that the proliferation of the lenticels of potatoes is increased in wet soils, and it is quite possible that there is a close correlation between early lenticel growth in dry soils and susceptibility to the scab pathogene.

The possibility of the growing tuber exerting a chemical stimulus on the spores of the scab organism, or on the germ tubes, was not overlooked. From repeated experiment there was no indication that the tissue of young or old tubers, or of potato leaves, stimulated the germination of the spores of *A. scabies*.

The writer (13,14) twice reported a critical period of susceptibility to scab infection. This occurred during the first ten days of tuber development. With a critical period for infection, the relation of soil moisture to scab development becomes very important. Young tubers may escape infection during this period if excessive soil moisture or other unfavorable factors inhibit the growth of the pathogene, and, likewise, an effective means of control must exert its greatest influence at this time.

Relation of Soil Reaction to Germination and Growth

Gillespie (4) concluded from a study of several cultures of *Actinomyces scabies*, that a pH of 5.2 is about the lower limit for growth. Slight development occurred in few instances at pH 4.8.

The effect of hydrogen-ion concentration on the growth and spore germination of the strain used in these experiments was determined. A wide range of pH was obtained by adjusting Czapek's synthetic dextrose-nitrate solution, as recommended by Karrer and Webb (7). Twenty cc. of sterile solution thus made up were measured into 200 cc. pyrex flasks, loosely plugged, then 1 cc. of spore suspension was added, and the flasks set to incubate at 26° C. Electrometric determinations were made of the initial reaction of each series, with and without inoculum, and this was repeated in ten days in the acid series, and in 48 hours in the alkaline series, in order to note any change in reaction. The solutions containing inoculum soon became alkaline, depending on the rate of growth. Notes were taken on the time required for growth to appear, its relative progress and final develop-

ment. Each series was in triplicate, and the whole experiment was repeated three times. The results are summarized in table 4.

Evidently pH 5.36 is about the limiting acidity for germination of the strain of *A. scabies* used in the test, since microscopic examination at the end of 20 days showed no evidence of spore germination at this reaction. Possibly other pathogenic strains of *A. scabies* have a different limiting pH value for germination. A few slow growing colonies appeared on the seventh day at pH 5.56. At pH 6.95 to pH 9.82[4]–9.05, visible growth occurred in 48 hours. For the first three days there was a constant tendency for earlier and better growth above than below pH 8.30, but after this period the cultures growing in the media with initial pH ranging from 6.48 to 8.68

TABLE 4.—*The estimated growth progress of Actinomyces scabies, at 26° C, on Czapek's solution at different hydrogen-ion concentrations.*

Culture	pH value of medium		Growth progress at various day intervals					
	Initial	After 10 days	2	3	4	5	7	20
1	4.52	4.50	0	0	0	0	0	0
2	4.70	4.70	0	0	0	0	0	0
3	4.87	4.86	0	0	0	0	0	0
4	5.21	5.20	0	0	0	0	0	0
5	5.36	5.37	0	0	0	0	0	0
6	5.56	5.83[b]	0	0	0	0	tr.	4[c]
7	5.69		0	0	tr.	1	2	6
8	6.08		0	1	3	7	8	8
9	6.48		0	1	4	8	10	10
10	6.85		0	2	6	9	10	10
11	6.95		tr.	2	6	9	10	10
		After 48 hours						
12	7.16		tr.	2	7	9	10	10
13	7.56	7.51	tr.	3	7	9	10	10
14	7.76	7.66	tr.	3	7	9	10	10
15	8.38	8.30	1	4	7	9	10	10
16	8.68	8.51	2	6	8	9	10	10
17	9.31	8.80	2	6	8	9	9	9
18	9.43	8.88	2	6	8	8	8	9
19	9.82	9.05	2	6	8	8	8	8
20	10.21	9.34	0	0	2	4	5	7

[a] 0 = no growth; tr. = trace; 1 = faint; 2 = plainly visible; 4 = fair; 6 = good; 10 = excellent.

[b] Determination made from growing culture.

[c] Few well developed colonies.

[4] The first figure is the initial reaction, the second that obtained after 48 hours.

seemed to have made more vegetative growth and to have fructified more abundantly than those above pH 8.68. Germination was slow and final growth poor above pH 10.2–9.34. No attempt was made to determine the maximum pH for germination because of the difficulty of holding the high alkaline solutions constant and the probable effect on growth of the large amount of potassium hydroxide. It would appear, however, that this point is not far from pH 10.

Available food, soil, aeration, moisture, and other biological factors, as well as soil reaction, must influence the amount of inoculum in the soil, and the amount of scab which develops. However, it does not appear that a soil reaction which is the limiting point for germination of the inoculum will completely inhibit development of scab. This might be because the growing tuber has a reaction favorable to germination and growth of *A. scabies*. Weiss and Harvey (19) found the reaction of the potato tissue to vary from pH 6.5 to 6.61. Consequently, the acidity about the closely adhering inoculum might be decreased enough to allow germination of the spores and infection of the host. If so, subsequent development would be largely determined by the reaction of the host tissue. Possibly this suggests why Martin (10) obtained scab on 20.6 and 89.6 per cent of the tubers in a soil of pH 4.2 and 4.8, respectively.

Weiss and Harvey found that tissue affected with potato wart was more acid (pH 6.0) than the adjacent sound tissue (pH 6.5). The writer determined electrometrically the reaction of scab pustules. Very scabby young potatoes grown in a soil having a reaction of about pH 5.4, were washed in distilled water, the scab pustules removed, finely pulverized, and the material diluted with distilled water. Sound tissue was treated likewise, and determinations made as soon as possible. The pH of the sound tissue was about 6.5, while that of the scab pustules was about 7.2. This increase in alkalinity agrees with what happens in liquid cultures and supports the previous suggestion that the development of *A. scabies* on infected tubers is largely independent of soil reaction.

The Relation of Green Crops to the Control of Potato Scab

The popular practice of plowing down green crops, particularly rye, to reduce the severity of scab is based largely on the assumption that decomposition of the green material increases soil acidity. Since consistent evidence for such belief seemed to be lacking, the writer tested the efficacy of the method in a well checked field experiment.

The effect of green rye plant material on soil reaction. A small, nearly level, thoroughly infested plot of fertile silt loam, with an initial pH ranging from 4.91 to 5.4, was chosen for the test. Twelve rows, of 12 hills each, were planted, test rows and controls alternating. Two and one half pounds

of succulent green material was mixed with the soil, and this put back into the excavations, which were 14 inches in diameter and 7 inches deep. This proportion of green material amounts to about 50 tons per acre. The soil in the control hills was forked to make it as loose as that under test. A disinfected set of the cobbler variety was planted 5 inches deep in each hill. The soil about each plant was not disturbed during the growth period. The rye was almost completely decomposed by harvest, 80 days after date of planting (May 24). The reaction of the soil, taken from representative hills at depths of 2, 4 and 6 inches, was determined electrometrically on June 19, July 2, and July 22. Samples were taken from the same hills each time, so that any change in soil reaction resulting from the decomposition of the green material might not be confused with soil heterogeneity. One hundred and thirty-two samples, of which 24 were checks, were tested, and three determinations made from each sample.

The object of this experiment was to note any change in soil reaction caused by the green rye during the early growth period of the potato crop, and particularly during the critical stage for scab infection. Therefore the reaction of the soil was not determined after 58 days. The data indicated

TABLE 5.—*The effect of varying amounts of green rye on the soil reactions and on the relative number of bacteria and fungi*

Parts of rye to soil	Interval after which pH was taken					Initial pH	Maximum pH increase	No.[a] colonies	
								Bacteria[b]	Fungi[c]
	Series 1								
	10 days	20 days	28 days	36 days	78 days				
	pH	pH	pH	pH	pH				
1–5	8.99	7.56	7.25	7.10	7.04		1.82		
check	7.22	7.20	7.13	7.14	7.14	7.17			
	Series 2								
	7 days	15 days	33 days	75 days					
	pH	pH	pH	pH	pH				
1–3	8.82	8.22	5.82	5.07			3.60	200	70
1–7.5	7.70	7.49	5.24	4.98			2.48		
1–10	7.37	5.76	5.37	4.97			2.15	134	33
1–15	6.89	5.73	5.24	5.24			1.67		
1–20	6.67	5.51	5.27	5.22			1.45	16	26
check	5.31	5.25	5.22	5.19		5.22			

[a] In millions per gram of rye soil mixture, oven dry.

[b] Dilutions made after 20 days.

[c] Dilutions made after 10 days.

a slight decrease in soil acidity. This conclusion agrees closely with that of Stephenson (16) who found that in nearly every case the acidity was decreased where soy beans, oat straw, green rape, and blood were used alone and in combination in the soil. Oat straw had the least effect in decreasing the acidity, while the highly nitrogenous material had the greatest.

Another study was made in the laboratory to determine the effect of the decomposition of green rye plants on soil reaction. A quantity of leaves from green rye plants was finely ground. This was mixed in varying amounts with finely sifted, unsterilized garden soil, then moistened with distilled water and put into loosely plugged liter flasks. All samples were approximately at optimum moisture content when completed, and the soil was loose in the flasks. The initial reaction of the soil, and also of the green material used, was determined electrometrically. The flasks were kept at room temperature and the soil reaction determined at intervals during 80 days. The number of fungi and bacteria developing in the soil of the various flasks was obtained by the usual dilution-plate method, and a comparison made on an oven-dry basis per gram of soil.

An analysis of the results in table 5 shows that the pH is decidedly increased in all the soils mixed with green rye-plant material, especially during the first ten days, and that this sudden rise is succeeded by a slow but steady decline during the second period. After about 30 days the pH values approach a level approximating the initial reaction. This is especially noticeable in the soils containing the smaller proportion of plant material. The increase in pH is, in general, proportional to the amount of plant material added to the soil, and varies from 1.45 to 3.60. In most cases, even after 30 days, this advantage is maintained. After 30 days, and continuing to 75 days, all the samples return to the initial reaction and, when the last determinations were made, only those samples receiving the larger proportions (1–3, 1–7.5, 1–10) of rye were slightly more acid than the untreated soils.

Since the determinations were made on the rye-soil samples after fermentation began, the increase due to the reaction of the rye itself was unknown; therefore a separate experiment was necessary to obtain this information. The initial pH of the green rye was 6.82, and that of the soil 5.12. The data show that the pH of the rye-soil mixture was increased very slightly by adding the green rye. The largest proportion of rye to soil (1–5) increased the pH 0.36, while the 1–10, 1–15, 1–20 proportions gave increases of 0.20, 0.18 and 0.17, respectively. After an 8-day incubation period the increases in pH of these same mixtures were: 1–5, 1.71; 1–10, 0.94; 1–15, 0.80; 1–20, 0.71. Apparently, then, nearly all the increase in pH of this and the previous test was the result of fermentation.

Two suggestions are given for the consistent increase in pH of the rye-treated soils; either the rye may favor certain microorganisms which decompose it and liberate ammonia for a time, or certain alkaline by-products of metabolism may be secreted by the fungi and bacteria themselves. Doryland (2) found that nitrification begins when the more easily available carbohydrate energy is exhausted, and states that the ammonia and nitrates which have been accumulated by the decay-producing organisms during this period are later available for nitrification. Waksman (18) states that the molds are not nitrifiers but they actively decompose organic matter, taking energy first from the available carbohydrates and then from protein molecules, in which case ammonia is liberated. He also points out how ammonia may be liberated upon autolysis of the mycelium of these molds. Neller (12) found that certain pure cultures of bacteria caused the accumulation of much more ammonia than some of the more active soil fungi (*Aspergillus niger, Trichoderma* sp., and *Zygorhynchus* sp.) and that there was an indication of symbiosis in mixed cultures of certain soil bacteria in increasing the accumulation of ammonia.

It is possible that the large amount of carbohydrate energy in the rye permitted abundant and quick growth of microorganisms, with a corresponding nitrate depletion of the soil, and that, upon the sudden exhaustion of the carbohydrate energy supply and the consequent setting in of the nitrification process, certain more favored bacteria and fungi actually cause the liberation of ammonia.

It was suggested that the increase in pH might be due to the alkaline by-products secreted by one or more predominating species of microorganisms. It has been shown that *A. scabies* greatly increases the pH of the culture media tested. It also is well known that other microorganisms produce acids and alkalies, depending on the chemical nature of the substrate.

A plate count of the microorganisms was made from dilutions of the soil of Series 2 (see table 5). In the dilutions made 10 days after mixing the rye and soil, the number of fungi on the plate made the count of the bacteria too uncertain to record, but, when made after 20 days, the plates were fairly free from fungi, and bacteria predominated. A heavy growth of molds (*Mucor racemosus* appeared to predominate) occurred in all flasks containing the rye-soil mixture. This took place two days after mixing; and in ten days it had declined, the soil taking on a moist, slimy appearance, which indicated bacterial activity. No further change was noted and no more soil dilutions were made. It is suggested that the fungal-bacterial succession referred to here may be associated with the rise and decline of pH noticed in these rye-treated soils.

The effect of green rye plant material on scab development. Figure 2 shows the amount of scab developing on the tubers in rye-treated and untreated soils of the field. Each pile represents the complete yield from a row, and these are arranged as they grew—treated and untreated rows alternating. No difference between the test lots and the controls can be noticed: all are badly scabbed. These results differ from those of Millard (11), who obtained good control by a liberal application of green rye; and, since the data on soil reaction could not explain the results, a preferential food theory was offered. This theory assumes that the scab pathogene, being normally a soil saprophyte, prefers green rye to the living potato tissue. The hypothesis may be tenable. The writer advances another possible explanation, and this will be referred to in connection with the antibiosis of certain bacteria and fungi to *A. scabies*.

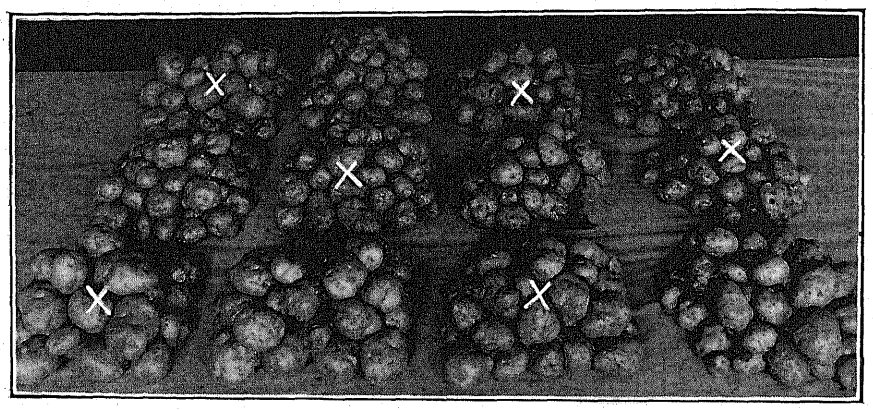

FIG. 2. The piles of tubers marked X are from soil (pH 5 to 5.4) treated with 50 tons per acre of green rye; remainder from untreated soil. Note uniformly large amount of scab in all piles.

The data from the field experiment showed that the decomposition of 50 tons of green rye per acre caused no noticeable increase in soil acidity during the 58-day period and failed to prevent the development of scab. The laboratory experiment showed that it required a much larger quantity than this to increase the acidity of the same soil, even slightly, at the end of 75 days. It is interesting to recall that the initial reaction of this soil varied from pH 5.0 to 5.4, which is very near the limiting reaction for growth of *A. scabies* in pure culture. The acid-producing effects of green rye on other soil types was not tested. However, these results strongly suggest that an abnormally large amount of rye would be required to increase the acidity of less acid soils to an effective point for scab control. Therefore it is the

opinion of the writer that the use of green rye as a practical means of increasing the soil acidity for the control of potato scab is not feasible. If control can be obtained by its use in some soils, it may be that certain microorganisms of the greatly increased soil flora are antibiotic to *A. scabies*.

Cultural Relations of Certain Bacteria and Actinomyces scabies

While testing the stimulatory effects of potato leaf- and tuber-tissue on the germination of the spores of *A. scabies*, it was frequently observed that the spores failed to germinate on certain slides overrun with bacteria. Further examination showed that the lack of germination was often associated with the kind of bacteria present, therefore possibly a result of toxicity or a too highly acid condition.

Effect of mixed cultures on the pH of the substrate. To study the effects of association of bacteria and *A. scabies* in culture, a number of pure cultures of bacteria were secured from various sources, chiefly from the soil. These were numbered as represented in table 6. Culture 21 is *Bacterium fluorescens;* 28, *B. mycoides;* 24, *B. cereus.* These were obtained from H. J. Conn, New York Agricultural Experiment Station. Culture 22 is *B. vulgatus;* 23, *B. megatherium;* 25, *B. mycoides;* 27, *B. mesentericus,* all of which were furnished by O. B. Williams, University of Texas. Czapek's dextrose nitrate solution was used for a medium. This was sterilized and adjusted to the required pH values by the addition of one-tenth normal potassium hydroxide. Pyrex flasks were used, and to each was added 20 cc. of the medium, and inoculum consisting of 0.1 cc. of a spore suspension of *A. scabies,* and the amount of bacteria adhering to the small loop of a transfer needle. Each culture was made in triplicate and incubated at 26–27° C.

The results given in columns 2 and 7, table 6, indicate that nearly all the strains of bacteria are strong acid producers. The reactions listed in column 2 give an idea of the acid-producing ability of each pure culture of bacteria when grown alone. Cultures 21 and 29 are fairly strong acid producers, although in Series 1 the time during which they were incubated is too short to ascertain the final effect in this respect. The reactions in column 6 indicate the strong alkali-producing ability of *A. scabies* during six days, when the initial pH of the medium is 7.3. This quality is also shown, in columns 9 and 11, in those cultures where the Actinomycetes are growing vigorously, apparently having secured control by neutralizing the acid from the bacteria. Evidently *A. scabies,* when grown alone or with certain bacteria, grows and fructifies exceptionally well at pH 8.0 to 8.7. Most of the cultures of bacteria allow a good final growth of *A. scabies* if the initial pH of the medium is sufficiently high. Bacterial cultures 11, 15, 21 and 29, however, always prevent the growth of the Actinomycetes. In the case of

cultures 11, 21 and 29, the growth is prevented by a too acid condition of the medium, *i.e.*, pH 4.23, 4.43 and 5.29 respectively. These cultures have reduced the pH from 7.3 to these low values in six days, while most of the other strains allowed the Actinomycetes to increase it to about 8.7. Culture 15 is outstanding, because it always prevents the growth of *A. scabies*,

TABLE 6.—*The effect of pure cultures of bacteria growing alone, and also with A. scabies, on the pH value of Czapek's solution*

Culture no.	Series 1					Series 2				
	Bacteria initial pH 7.3	Bacteria and *A. scabies* initial pH 7.3				Bacteria initial pH 6.87	Bacteria and *A. scabies* initial pH 5.7			
		Growth: after 3 days	pH	Growth: after 6 days	pH		Growth: after 6 days	pH	Growth: after 11 days	pH
1	6.53	Good	7.41	Ex.[a]	8.67	.....	.....	.....	.....	.....
2	6.44	do	7.68	do	8.72	.....	None	5.02	None	5.41
4	6.12	do	6.48	do	8.75	.....	.....	.....	.....	.....
5	6.13	do	7.12	do	8.73	.....	None	4.91	None	5.59
8	6.61	do	7.33	do	8.72	.....	.....	.....	.....	.....
10	6.99	do	7.67	do	8.69	.....	.....	.....	.....	.....
11	6.05	Trace	4.34	None	4.23	.....	None	4.77	None	4.57
12	6.86	Good	7.39	Ex.	8.73	.....	do	5.40	Ex.	8.04
13	5.67	do	7.80	Good	8.72	.....	.....	.....	.....	.....
15	6.78	None	6.63	None	7.28	.....	None	7.35	None	7.29
16	6.44	Trace	6.44	Ex.	8.72	.....	Trace	4.89	do	4.22
17	6.61	do	6.95	do	8.59	.....	None	4.57	do	4.60
19	6.30	Good	7.14	do	8.57	5.51	do	5.32	Good	8.14
20	6.89	do	7.17	do	8.57	.....	.....	.....	.....	.....
21	6.71	None	5.11	None	4.43	4.30	None	4.73	None	4.80
22	6.61	Good	7.49	Ex.	8.72	.....	.....	.....	.....	.....
23	6.81	Trace	5.43	Faint	7.36	.....	None	4.92	Edge	5.41
24	6.53	Good	7.61	Ex.	8.72	6.16	do	5.29	do	6.41
25	6.71	do	7.21	do	8.72	6.14	do	4.81	None	4.73
26	6.64	do	6.97	do	8.70	.....	.....	.....	.....	.....
27	6.69	Trace	7.39	do	8.79	5.66	None	4.89	None	5.03
28	6.53	Good	7.26	do	8.89	5.92	do	4.72	do	4.72
29	6.66	None	5.26	None	5.29	5.34	do	4.83	do	4.64
30	6.99	Edge	7.56	Pel.[b]	7.80	.....	Pel.	7.35	Edge	7.63
31	6.71	Fair	7.22	Fair	7.46	.....	None	5.27	Ex.	8.04
32	7.05	Good	8.52	Ex.	8.72	.....	.....	.....	Pel.	6.07
33	6.66	do	8.01	do	8.72	.....	Pel.	7.06	Edge	7.70
Check	7.70	do	8.51	do	8.72	.....	Fair	7.06	Ex.	8.40

[a] Excellent.
[b] Pellicle.

although it does not reduce the pH below a point favorable for growth of the fungus. When the initial pH of the medium is low (5.7), as in Series 2, many of the cultures of bacteria retard or prevent the growth of *A. scabies* by quickly increasing the acidity of the substrate, but some do not produce acid fast enough to accomplish this. This competition between the bacteria and the Actinomycetes makes the final outcome uncertain, as is shown by cultures 2, 5, and 23, in columns 9 and 11.

Further examination of table 6, Series 1, shows that some strains of bacteria increase the acidity of the medium much more when cultured with *A. scabies* than when alone. This action is well illustrated by cultures 11, 15, 21 and 29, in figure 3 and particularly by cultures 11, 21 and 29. The decrease in pH, as a result of association, ranges from 0.95 to 1.71. A parallel case is reported by Neller (12), who found that *Bacillus mycoides* and *B. vulgatus* produced more ammonia in mixed cultures than when alone, and that the same thing was true of the production of carbon dioxide by *B. subtilis* and *B. vulgatus*. Other instances of increased activity arising from the association of certain microorganisms are known.

Toxicity and compatibility in mixed cultures. Grieg-Smith (5) states that certain white-growing colonies of Actinomycetes secreted some product very toxic to *Bacillus vulgatus, B. mycoides* and also to *Penicillium* spp. and *Aspergillus* spp., and that the dark-growing[5] Actinomycetes were only feebly toxic to *B. vulgatus*. The writer has observed that *A. scabies* is, in general, very sensitive to the secreted products of many molds and bacteria, some of which prevent its growth. *Rhizopus* spp. have been consistently very toxic. Species of *Penicillium* and *Aspergillus* usually appear to be toxic to *A. scabies,* but this condition often develops later into one of compatibility, especially on solid media. Some of these cases of toxicity are probably associated with competition for food and with an unfavorable substrate reaction. The competition for favorable substrate reaction at the minimum point for growth is well illustrated by *A. scabies* and bacterial cultures 19 and 24, Series 2, table 6, and in figure 3, part 2. Bacterial culture 15 evidently secretes another kind of toxic substance, which, while not too acid, prevents the growth of *A. scabies.*

Further investigation of the association of *A. scabies* and the various strains of bacteria cultured in solution (table 6) was made on solid media. Potato dextrose agar slants were evenly inoculated with 0.2 cc. of spore suspension of *A. scabies* and then inoculated for 24 hours. By that time growth was quite evident. Then 0.2 cc. of sterile distilled water was added to the very bottom of each tube, and this was inoculated with a small amount of bacteria adhering to the loop of a transfer needle. Care was taken to add

[5] *Actinomyces scabies* belongs to this group.

nearly equal quantities of the bacterial inoculum to the water at the bottom of the tube only, and to keep the tubes upright. Striking differences in

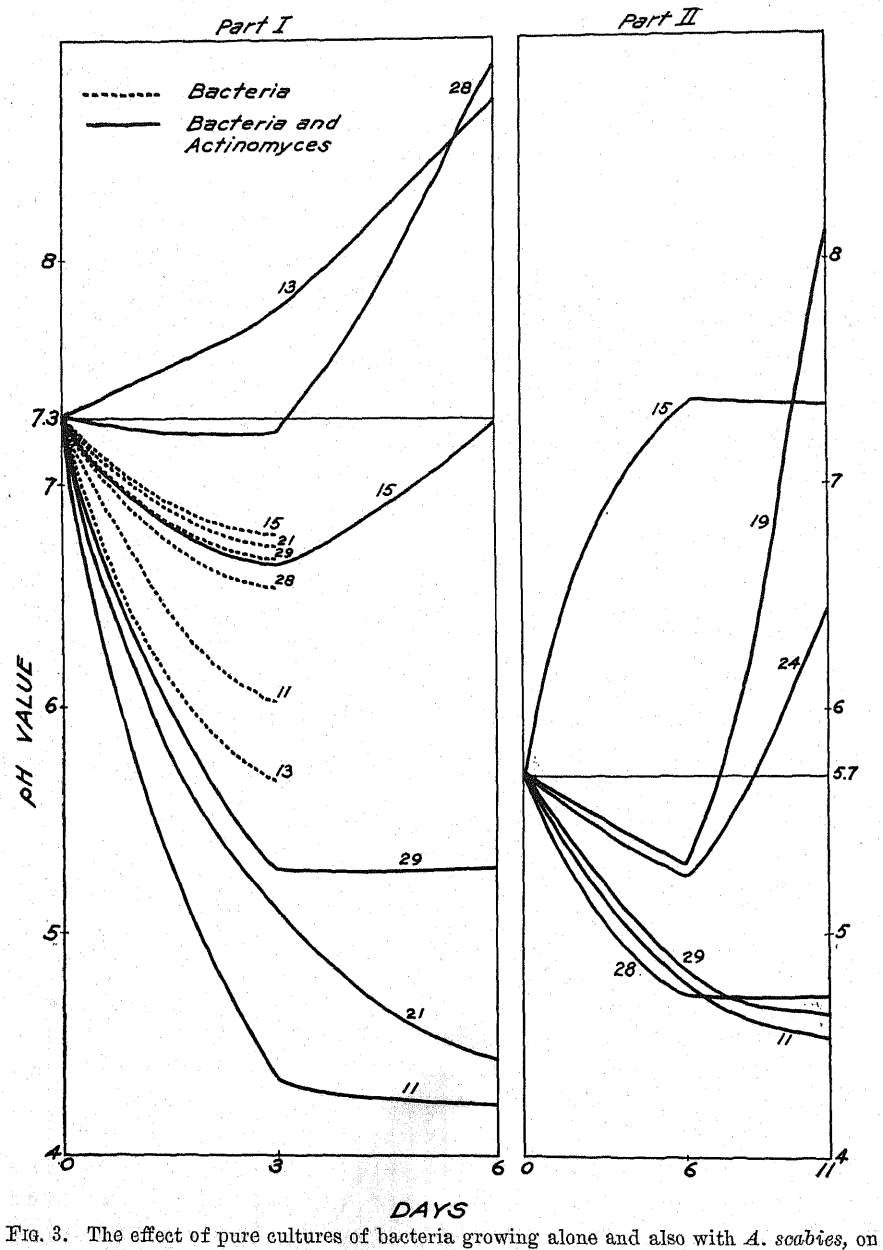

FIG. 3. The effect of pure cultures of bacteria growing alone and also with *A. scabies*, on the pH value of Czapek's solution. (Each culture is designated by the number just above the pH curve; the numbers correspond with those in table 6.)

growth were evident in a few days. A number of the strains seemed more or less compatible. As happened in the liquid media, bacterial strains 11, 15, 21 and 29 were sufficiently toxic to *A. scabies* to destroy the growth after it was visible. Cultures 2, 5, 12, 28 and 30 also were very toxic, although they had not shown this in the liquid media. The phenomenon is not unlike the activity of a lytic principle.

It is quite possible that the same degrees of compatibility between organisms and the same toxicity demonstrated on artificial media may also occur naturally in the soil. This is a problem of the biological balance in the soil and therefore may be of particular importance in determining the pathogenicity of *A. scabies*.

SUMMARY AND CONCLUSIONS

1. A study has been made of the effect of temperature, moisture, aeration, and substrate reaction on the germination, growth and fructification of *Actinomyces scabies*, with particular reference to its pathogenicity. The relation of green rye to the control of potato scab has been studied, and its effect on soil reaction and on the increase of soil flora has been observed. A brief study has been made of some cultural relations between *A. scabies* and some common soil bacteria.

2. *A. scabies* grows within a wide range of temperature (8 to 38° C). Good growth and maturity occur between 13° and 32° C., and the optimum temperature is about 27° C. Therefore, under average field conditions in most potato growing areas, it appears that temperature, as it affects host and pathogene only, cannot be a very important factor in the scab problem. The spores survive temperatures up to 90° C. (moist heat) for ten minutes.

3. *A. scabies* is a strong aerobe. The spores will germinate with an extremely small supply of oxygen, but a large amount is required for subsequent development. Maturity, as indicated by dark aerial hyphae, will not take place in the absence of oxygen. Amount of oxygen, not partial pressure, is the limiting factor for germination and growth.

4. It was found that the germination of spores of *A. scabies* on nutrient agar was greatly retarded by a lagging film of excess water. The inoculum of *A. scabies* appeared to increase best at a soil moisture content about optimum for plant growth.

5. The limiting acid reaction for germination of the spores of the strain of *A. scabies* used was found to be about pH 5.3. Germination occurred most quickly at about pH 8.5, and an optimum development took place at this point. Severe scab may be expected in soils ranging from a strongly alkaline reaction to at least pH 5.4, owing to the higher pH of the tuber and a strong tendency of the pathogene to make its habitat (scab pustule) alkaline.

6. Green rye plants, applied in the field at the rate of 50 tons per acre, showed no tendency to reduce scab in a thoroughly infested soil of pH 5 to 5.4; and there was no noticeable increase in the soil acidity during a 58-day period.

7. In the laboratory there was a very slight increase in acidity of the rye-treated soils after 75 days, but only in those receiving large proportions (1–3, 1–7.5, 1–10) of green rye. These results, and those obtained in the field, indicate that the use of green rye does not control potato scab by increasing the soil acidity.

8. When pure strains of bacteria and *A. scabies* were cultured in the same solution, certain strains of the former made the solution too acid for the germination of the latter. Certain cultures of bacteria produced more acid when grown with *A. scabies* than when grown alone.

9. A strain of bacteria isolated from the soil, and cultured in Czapek's solution, inhibited the development of *A. scabies* by some means other than excessive acidity.

10. Nearly all the bacterial strains used in this study made conditions more or less unfavorable for the growth of *A. scabies* on a potato-dextrose agar.

11. When scab is controlled by green rye crops on some soils, it is suggested that the antibiotic qualities of certain predominant soil microorganisms influence the development of *A. scabies*.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
ST. PAUL, MINNESOTA

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BLACK-LEAF OF PEAS CAUSED BY FUSICLADIUM PISICOLA N. SP.

MAURICE B. LINFORD[1]

In the summer of 1921 a leaf-spot disease was observed in fields of canning peas (*Pisum sativum* L.) at Smithfield, Cache County, Utah, where it was causing considerable leaf destruction in peas at canning age. Although peas had been grown in this locality since 1918, this disease had not been reported before. The disease was clearly distinct in appearance from any known to affect peas, being characterized by spots of sooty blackness on the lower sides of leaves and on petioles and tendrils. Microscopic examination revealed the presence of two fungi or two sporulating forms, one a *Fusicladium* bearing conidia on the lower side of the leaf, the other a *Phyllosticta.*

DESCRIPTION OF THE DISEASE

The first symptom of black-leaf is the development on the lower surface of leaflets and stipules of small, irregular, whitish areas, from 1 to 7 mm. across (Pl. XXVI, A), almost simultaneously with the appearance of slightly pale areas on the upper side. These whitish areas become somewhat larger and then, developing a tan color at the center, darken centrifugally through shades of gray to black. This darkening of the spot is due to the development of a layer of closely packed conidia on the leaf surface. After thus blackening, the center of the spot may grow lighter, changing to gray or olive gray when the conidia have fallen. Spots are limited laterally by the primary and secondary veins, which they seem unable to cross. Longitudinally spots may increase indefinitely, in which case they may be gray at the center, black where fresh conidia stand compactly, and whitish at the advancing margins (Pl. XXVI, A and B). Spots may become confluent near the margins of a leaflet where veins are small. Blackening is not always continuous in an affected spot but may assume an irregularly patchy or reticulate pattern (stipules, Pl. XXVI, A).

Development of this black conidial layer on the lower side of the leaf is not usually followed by collapse of the tissues until after the conidia are

[1] The writer wishes to acknowledge his indebtedness to Professor B. L. Richards, of the Utah Agricultural College, at whose suggestion this work was undertaken, for advice and encouragement throughout the course of this study; also to Dr. J. J. Davis, of the University of Wisconsin, who has aided in the taxonomic study.

shed. The spots then dry to brittleness and may crack out (Pl. XXVI, C). In such late stages a spot may be black above as well as below. In exceptional cases a few conidia are borne on the upper face of a diseased spot. Plate XXVI, D and E, shows the typical appearance of an old spot as seen from the upper and lower sides, respectively.

After conidia-bearing spots have stopped enlarging and most of the conidia are shed, a bluish gray color appears around and sometimes within the conidia-bearing areas. This results from the development, beneath the epidermis, of numerous minute, black, pycnidium-like bodies, more numerous below, but not absent from the upper side of the leaf. Commonly the pycnidial zone is narrow at the margins of old conidial spots, but at times large areas of a leaf may be involved. The pycnidium-like bodies have been observed in material collected in many localities. In no instance have they been observed in a leaflet that had not previously borne conidia. As with the conidial areas, collapse of the tissues usually does not follow promptly the development of pycnidia; the invaded areas are not margined.

The black-leaf disease develops less abundantly and more tardily on petioles and tendrils than on leaflets and stipules, but in the later stages it is not uncommon to find tendrils completely girdled and petioles bearing elongate spots. A spot on a petiole, even if it girdles, does not result in prompt killing of the leaflets; there seems to be no direct interference with the conductive tissues. As a rule production of conidia is reduced, and the production of pycnidia is relatively more abundant than on the laminae.

Black-leaf has rarely been observed in a field before the peas approach full blossom. Developing on the lower leaves at first, spots appear progressively higher on the plant until, when the peas are ready for canning, spots are generally developed even on the uppermost leaves (Pl. XXVI, F).

VARIETAL SUSCEPTIBILITY

No comparative trials of varieties have been made, but the black-leaf disease has been observed on the following varieties:

White (Yellow) Admiral	Green Admiral
Horal	Alaska
Champion of England	Sugar Marrowfat

Of these the White Admiral appears to be the most susceptible. In Utah the disease has been reported only from canning factory districts in which this is the dominant variety grown. In several districts where the disease has been sought in vain, this variety is not grown. In a number of instances fields of Alaska and White Admiral were seen growing side by side; in all such cases the disease, if present, was most abundant in the Admiral. In

Idaho the experience of a prominent seedsman indicates that the White Admiral is more frequently affected than any other variety.

PRESENT DISTRIBUTION

Black-leaf is known at present to occur only in three valleys in northern Utah and in the great seed pea producing area in the Snake River Valley of Idaho, from Idaho Falls to Ashton. In the Cache, Ogden, and Weber valleys of Utah the disease has been observed each year since it was first seen in 1921, varying greatly in severity. During the dry summer of 1924 it was all but absent. In Idaho the writer has seen black-leaf only in the summer of 1925. One of the leading seedsmen, however, described black-leaf in an unmistakable manner as a disease affecting the White Admiral pea in wet seasons in that region for the past ten years.

ECONOMIC IMPORTANCE

Black-leaf is probably not greatly to be feared as a factor limiting pea production in the intermountain region, judging from the behavior of the disease observed during five seasons. Occasional fields have been found, especially in wet seasons, in which more than 50 per cent of the leaf surface has been destroyed by the time the crop has reached the stage for canning. In such cases there has been a marked reduction in yield, but generally the disease causes only moderate foliar destruction at so late a date that losses are negligible. In seed fields, owing to the longer time the crop must remain in the field, the disease may cause somewhat greater reductions of yield, but it has not been looked upon as of serious importance by seedsmen or growers.

MICROSCOPIC STUDIES

Microscopic studies of sections through young spots on leaves collected when symptoms first become discernible reveal the presence of a slender, sparingly branched, septate mycelium growing through the intercellular spaces of the leaf. Hyphae may be seen lying in intimate contact with host cells and extending the length of palisade cells. No haustoria have been detected. There appears to be no immediate reaction of host cells to the presence of these hyphae, but there is a gradual breaking down of the protoplasm, which finally disappears, leaving the cell walls intact. Except in the process of conidia-formation, the mycelium is strictly intercellular. When pycnidia commence to form, the mycelium becomes thicker, more conspicuously septate, and brownish in color. The pycnidia develop from hyphal knots in the intercellular spaces and gradually increase in size, crowding back the empty host cells. Thickened hyphae may fill the intercellular spaces, but as a rule the host cells retain their shape even after they are dead

(Pl. XXVII, A). Early death and collapse of some spots has been observed to inhibit the normal development of conidia and especially of pycnidia.

The apparent inability of the parasite to cross the larger veins of a leaf may be accounted for by its strictly intercellular habit. Above and below the larger veins is a ridge of collenchyma in which intercellular spaces are almost absent. In leaf sections hyphae may be seen advancing far into the available spaces but unable to make their way across such veins. In tendrils and petioles the distribution of intercellular spaces markedly restricts the advance of the fungus. As shown in Plate XXVII, B, the sub-epidermal layers of parenchymatous chlorenchyma in which large intercellular spaces are present are thoroughly invaded, but the more compact interior tissues remain free from invasion. Probably the failure of black-leaf to develop on pea stems is in part associated with the smaller size and extent of intercellular spaces in the sub-epidermal layers as compared with corresponding layers of the petiole.

DEVELOPMENT OF CONIDIA

Shortly after mycelium has appeared in the intercellular spaces of a leaf, hyphae may be seen growing in close contact with the cells of the lower epidermis. From such hyphae branches arise at right angles and grow directly through the inner wall of the epidermis into the epidermal cells. Swelling slightly, they continue to elongate until they push out through the outer wall, penetrating apparently by mechanical means. Protruding from the lower epidermis, these branches then enlarge into conidiophores which are clavate while young and are filled with dense cytoplasm. They are for a time non-septate and uninucleate, the large nucleus remaining near the base within the epidermal cell. A single bud-like projection then appears at the apex and proceeds to enlarge. It may be smoothly ovoid or may be elongate, marked early by a slight constriction at the place where septation may later develop. As the young conidium approaches mature size, the nucleus from the base of the conidiophore passes upwards, divides, and one daughter nucleus passes into the conidium. Septation by constriction then separates the conidium from the conidiophore. The nucleus in the conidium may or may not divide. In case of division the daughter nuclei separate and a septum is formed between them, developing inward from the side walls. The nucleus and cytoplasm remaining in the conidiophore collect in the upper end where they gradually degenerate and disappear. At the same time the upper part of the conidiophore wall becomes thicker and dark brown. By the time the conidium is shed the conidiophore is usually an empty conical structure, broad at the base, the wall of the upper part thickened until it almost closes the lumen, and the extreme tip almost black.

Functioning conidiophores have not been observed to pass through stomata or guard cells. In early stages of the disease hyphal branches may be observed to have grown out through stomatal openings. Such hyphae, as shown by staining reaction, have died after having extended only a short distance (4–10 μ) through the pore, showing as heavily staining clavate hyphae with no indications of the formation of conidia.

The young conidium may early be seen to have a double wall, the inner homogeneous in appearance, staining lightly; the outer dark brown and warted, staining deeply. The outer wall does not become thick, but its warted nature becomes more pronounced at maturity.

Mature conidia (Pl. XXVII, C) are typically ovate to cylindrical, constricted at the septum, rounded at the ends, and flattened at the point of attachment to the conidiophore. The lower cell is characteristically somewhat the larger. Shape varies with compactness of sporulation, spores borne in crowded masses being more slender than the type. The color in mass is dark olivaceous brown to black; under the microscope, a dilute brown.

TAXONOMY

The shape, septation, color, and mode of production of conidia place the fungus in the hyphomycetous genus *Fusicladium* Bonorden. This fungus differs from other species of this genus and bears some resemblance to *Hetcrosporium* Klotzsch in the roughening of the spore wall, but no spores have been observed with more than one septum. Recently von Höhnel (3) has proposed a revision of the genera *Fusicladium* and *Passalora,* suggesting a number of new genera. Since the advisability of this revision is questioned and since none of the newly defined genera appear to include this fungus, the genus as defined by Bonorden (1) is here accepted.

Only two species of *Fusicladium* are known to have been listed as occurring on peas. Oudemans (6) lists *Fusicladium pisi* Oud. and refers to an earlier paper (4) for the description. In this earlier paper there is no mention of this binomial. Instead, *Fusicladium fagopyri* Oud. is described as occurring on the leaves of peas. Oudemans, in his later work (5), states that he had been in error in listing *F. fagopyri* as occurring on peas. Thus the binomial *Fusicladium pisi* Oud. stands in literature with no description. As for *F. fagopyri,* Oudemans's description does not fit the fungus under present consideration; his spore measurements of 14×9 μ are less than the minimum dimensions of the septate spores, and his measurements of conidiophores are several times longer than the conidiophores of this fungus.

Fusicladium brevipes Ell. and Ev. was described in 1889 (2) from specimens collected in the Sangre de Christo mountains of Colorado, occurring on *Astragalus hypoglottis.* The fungus on peas was tentatively but erroneously considered to be this species and was so reported in the Plant

Disease Reporter (7). Type specimens of this fungus from Colorado have been examined, through the kindness of Dr. Seaver of the New York Botanical Garden, and it appears to be entirely distinct from the fungus on peas. The spores are much longer, frequently biseptate, and have smooth walls. No other species of this genus are known to occur on plants closely related to *Pisum sativum*. The present fungus is therefore designated a new species, described as follows:

Fusicladium pisicola n. sp.

Mycelium intercellular in leaf parenchyma; spots dark gray, olivaceous, or black, immarginate, irregular to oblong, restricted in lateral extent by the larger veins, hypophyllous or rarely amphigenous; conidiophores growing through the epidermis, short, erect, continuous, conical, the walls thickened and dark brown at the truncate apex, 10–24 μ long and 4–6 μ broad above; conidia solitary on tips of the conidiophores, ovate to cylindrical, rounded at ends or truncate at base, generally becoming uniseptate, constricted at the septum, the cells equal or the lower somewhat larger, walls with fine wart-like roughening, brown, 16–32 × 8–14 μ.

On leaflets, stipules, tendrils, and petioles of *Pisum sativum* in Utah and Idaho.

THE PYCNIDIAL FUNGUS

The bodies which develop around and sometimes within the conidial areas may be seen under the microscope to be of two distinct classes: one a pycnidium, the other a sclerotial body. The sclerotial bodies are always in the majority; in fact pycnidia are often very rare. The pycnidia are spherical to ovoid with thin but distinct walls, averaging 60 × 70 μ. At first sub-epidermal, they finally become erumpent. Within the pycnidia, numerous, minute, hyaline, continuous, rodshaped spores are produced from the tips of pointed conidiophores which line the cavity. The spores are variable in size and are often poorly developed, attaining a maximum size of about 1.3 × 5 μ. This fungus is evidently a *Phyllosticta*.

Although several species of *Phyllosticta* have been described on *Pisum sativum* and related hosts, all seem different from this fungus either in size of spores or in the nature of the spot produced. Since, however, its relationship to *Fusicladium pisicola* remains uncertain, no specific name is given at present.

The sclerotial bodies average somewhat larger than the pycnidia, ranging from 75 to 90 μ in diameter. Resembling the pycnidia very closely in shape, they differ in having a slightly thicker wall and in being composed of a mass of interwoven, loosely packed, irregular, hyaline mycelium. No spores of any kind are associated with these bodies during the summer, but some indications suggest that they represent a stage in the development of

perithecia. That the sclerotial bodies are produced by the same fungus as develops the pycnidia is shown conclusively by the occurrence of occasional compound structures surrounded by one continuous wall, one-half being of typical pycnidium formation and the other half having typical sclerotial structure.

No such positive evidence is at hand concerning the relation of these structures to the *Fusicladium*. Microscopic evidence as well as field observations tend to indicate that the pycnidia, sclerotial bodies, and conidia are all associated with the same fungus, but only pure culture methods or inoculation studies can positively determine the connection.

OTHER FUNGI

While other fungi have been observed associated with black-leaf spots, none has been found with any degree of constancy in the earlier stages of development. The only one of these worthy of mention is a species of *Cladosporium* which appears as a common saprophyte on pea vines in the localities studied. This fungus is found sporulating in old conidial spots where it helps to produce the characteristic olivaceous color of old spots. The spores, catenulate, olive green, finely echinulate, pointed at each end, and continuous to biseptate, are readily distinguished from the conidia of *Fusicladium pisicola*. This fungus appears to hasten the collapse of invaded spots and is frequently present when pycnidia and sclerotia are commencing to form, but it probably has no connection with them.

ATTEMPTS TO CULTURE THE PARASITE

During three successive summers attempts were made to grow the *Fusicladium* in culture but without success. Dilutions of conidia were made in various ways. Spores were sown on and in various agars, and single-spore transfers were made to a variety of media. Spores from different localities and from young and old spots were tried, some fresh from the field and some that had been in storage for various lengths of time. The media used include water agar, prune agar, raisin agar, potato agar (with and without dextrose), nutrient agar (with and without dextrose), oatmeal agar, pea vine agar, cornmeal, steamed pea vines, pea vine decoction, and raisin decoction.

Conidia germinate freely on most of these media, producing germ tubes which reach their maximum development within 48 hours, seldom exceeding 140 μ in length. Single-spore transfers have been observed for as long as two weeks without further growth being detected. Besides dilution cultures, direct leaf-fragment platings were made in various ways, not only from conidial spots but from pycnidial areas as well, but with no better results. In all these attempts, *Cladosporium* was the fungus most frequently

obtained, but in no case did a spore marked as typical of *Fusicladium pisicola* produce a culture of *Cladosporium*.

FACTORS INFLUENCING GERMINATION

Conidia of *Fusicladium pisicola* were found to germinate only rarely in distilled water and poorly on water agar, a small percentage of spores giving rise to tubes apparently lacking in vigor. In prune and raisin decoctions much higher percentages of germination and stronger germ tubes were observed.

Temperature was observed to have a marked influence upon both percentage of spores germinating and type of germ tube produced. Two suspensions of conidia in prune decoction were divided into three lots, and one lot of each was held at temperatures of 7°, 17°, and 27° C. After 18 hours the percentages of spores germinated were as follows:

	7° C.	17° C.	27° C.
Sample A	50 per cent	66 per cent	25 per cent
Sample B	24 per cent	40 per cent	28 per cent

Not only did a higher percentage of spores germinate at 17° C. than at the other temperatures but the germ tubes attained greater maximum length. At 7° C. germ tubes were short and thick with a strong tendency to branch, while at the highest temperature germ tubes were very slender and with no branching. Those produced at 17° C. showed an intermediate thickness and but little branching.

Spores were applied dry, in drops of water, and in drops of dilute prune decoction, to the surface of green pea leaves under sterile conditions in petri dishes kept moist with wet filter paper. In all cases germination was rapid and vigorous. Leaf fragments bearing these germinating conidia were examined after being cleared in absolute alcohol-glacial acetic acid mixture but no tendency towards penetration was observed.

The length of time that spores have been found to remain germinable is variable. Some collections have shown prompt decline in germinability, while one collection germinated well in April, after having been in the herbarium nine months.

INOCULATION EXPERIMENTS

A number of inoculation experiments have failed to produce infection. Conidia have been applied to leaves of the White Admiral pea in various stages of development under a variety of conditions: applied dry and suspended in water; with and without first rubbing the bloom from the leaf surface; with and without injury to the epidermis. Several devices have

been used to maintain humidity. Introduction of conidia-bearing vines into plots of healthy peas has resulted in no infection.

DISCUSSION

The occurrence of a disease of some importance in a seed growing area from which great quantities of seed peas are shipped to the major pea canning regions of the country may be of some concern to users of this seed in districts where the disease has not appeared. While it is not yet possible to say with certainty whether this disease may be disseminated with pea seed, this seems unlikely. Black-leaf is not known to affect either the seeds or the pods. Any transfer of the fungus would therefore be only as spores adhering to the seeds. The chance of conidia borne thus on the seed being able to survive and to establish infection seems very small.

Until more is known of the life history of the parasite and of its relation to environment, it is impossible to say just what has limited the disease to the intermountain region. That black-leaf does not occur at present in Wisconsin seems certain: the writer, in conducting an intensive survey of diseases of canning peas in that state during 1924 and 1925, has been constantly on the alert for it. If, as pointed out earlier, black-leaf has been present in the seed districts of Idaho for a decade, it seems certain, in view of the great amount of seed annually shipped to Wisconsin, that either the disease is not seed-borne or that some factors inhibit its development in Wisconsin.

SUMMARY

1. Black-leaf of peas is a newly described disease characterized by dark olivaceous gray to black elongate spots on the lower side of leaves and on petioles and tendrils.

2. This disease is now known to occur in three valleys in northern Utah where peas are grown for canning, and in the great seed pea-producing area of the Snake River Valley, Idaho.

3. While black-leaf may cause considerable losses under favorable conditions, it has not been of great economic importance under usual conditions.

4. The earliest symptom of this disease is due to the development of conidia of a fungus described as *Fusicladium pisicola* n. sp. Associated with the older spots are pycnidia of Phyllosticta type and sclerotia of similar external appearance. The development of the fungus in the leaf is briefly traced.

5. The conidia germinate freely under a variety of conditions but do not continue their growth on the artificial media tried. The fungus has not been grown in culture. Preliminary inoculation studies have not established infection.

6. Positive evidence is lacking as to the possibility of black-leaf being introduced into new localities with seed from infested fields, and available information suggests that the chance of such introduction is slight.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
UTAH AGRICULTURAL COLLEGE,
LOGAN, UTAH.

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EXPLANATION OF PLATES

PLATE XXVI

A. Lower surface of leaf bearing numerous black-leaf spots in early and medium stages of development. The pale spot at 1 represents the earliest symptom of this disease. At 2 is a spot in which the central blackening is becoming apparent. ×2/3.

B. Group of spots at base of leaflet, showing lateral limitation by the larger veins and the terminal black bands where conidia stand compactly, marking the ends of spots that are elongating. ×1 1/3.

C. Late stages of the disease, showing cracking out of old diseased areas. The spots have become confluent near the leaf margin but are still separated by the larger veins towards the center. ×2/3.

D. Upper side of leaflet, showing typical appearance of a black-leaf spot in its later stages. Compare with E. ×1 1/3.

E. Lower side of leaflet, showing same spot as in D. Pycnidial bodies occur in a narrow band at margin of spot. ×1 1/3.

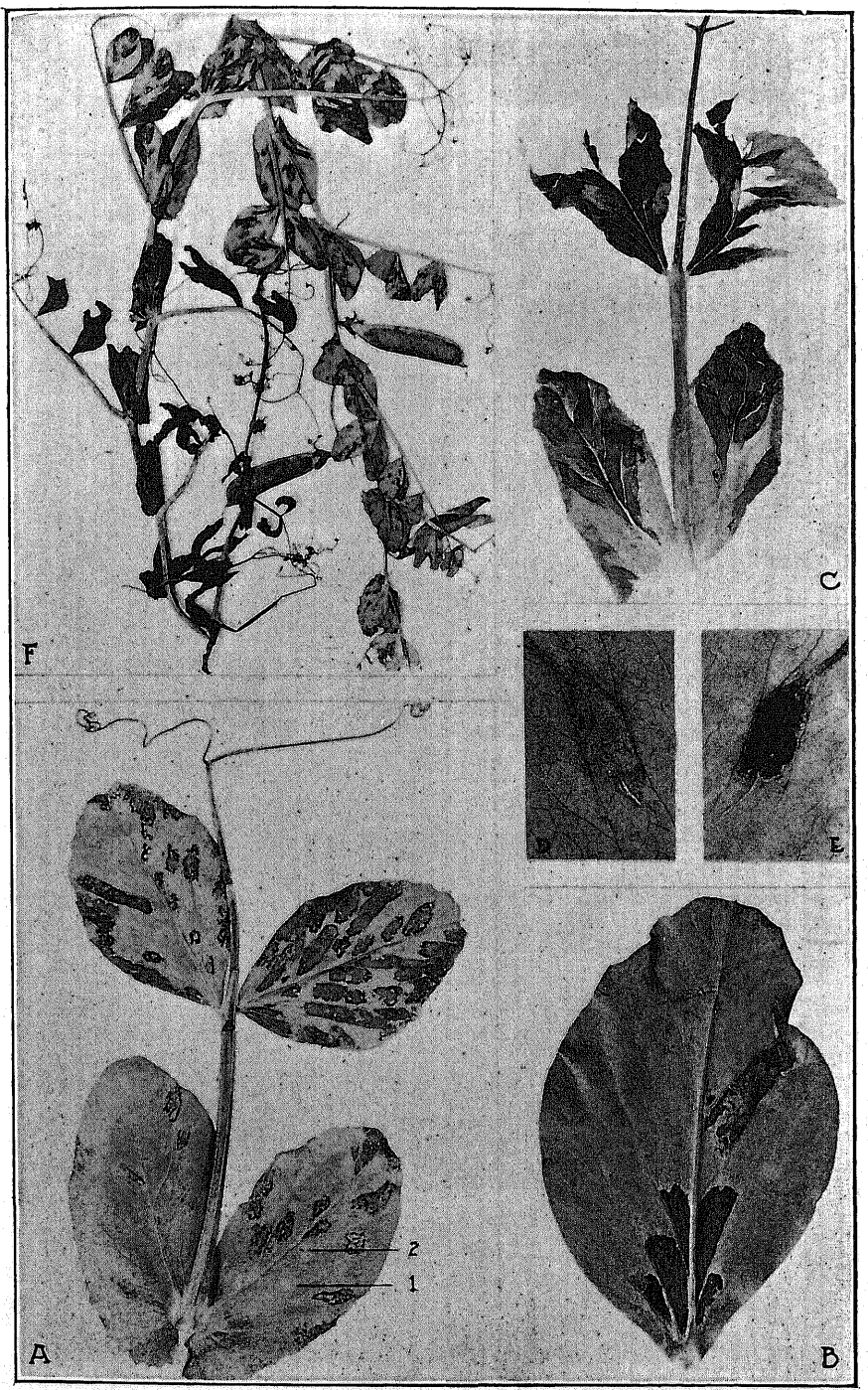
F. A plant at canning age showing heavy infection in late stages. The uppermost leaves are spotted but the pods are disease-free. The lower leaves have been killed. ×1/5.

PLATE XXVII

A. Transverse section through pycnidial area at margin of a conidial spot, showing at the right a sclerotial body and at the left a pycnidium filled with minute spores. The thickened mycelium is strictly intercellular, leaving the empty palisade cells (above) intact. ×500.

B. Portion of transverse section of petiole showing the limitation of the mycelium to the outer region in which large intercellular spaces are continuous. At the left the epidermis is roughened by the persistence of old conidiophores. Two sclerotial bodies are shown. ×150.

C. Conidial layer of *Fusicladium pisicola* at lower side of leaf, showing septate conidia borne apically on short conidiophores arising through the epidermis. × 500.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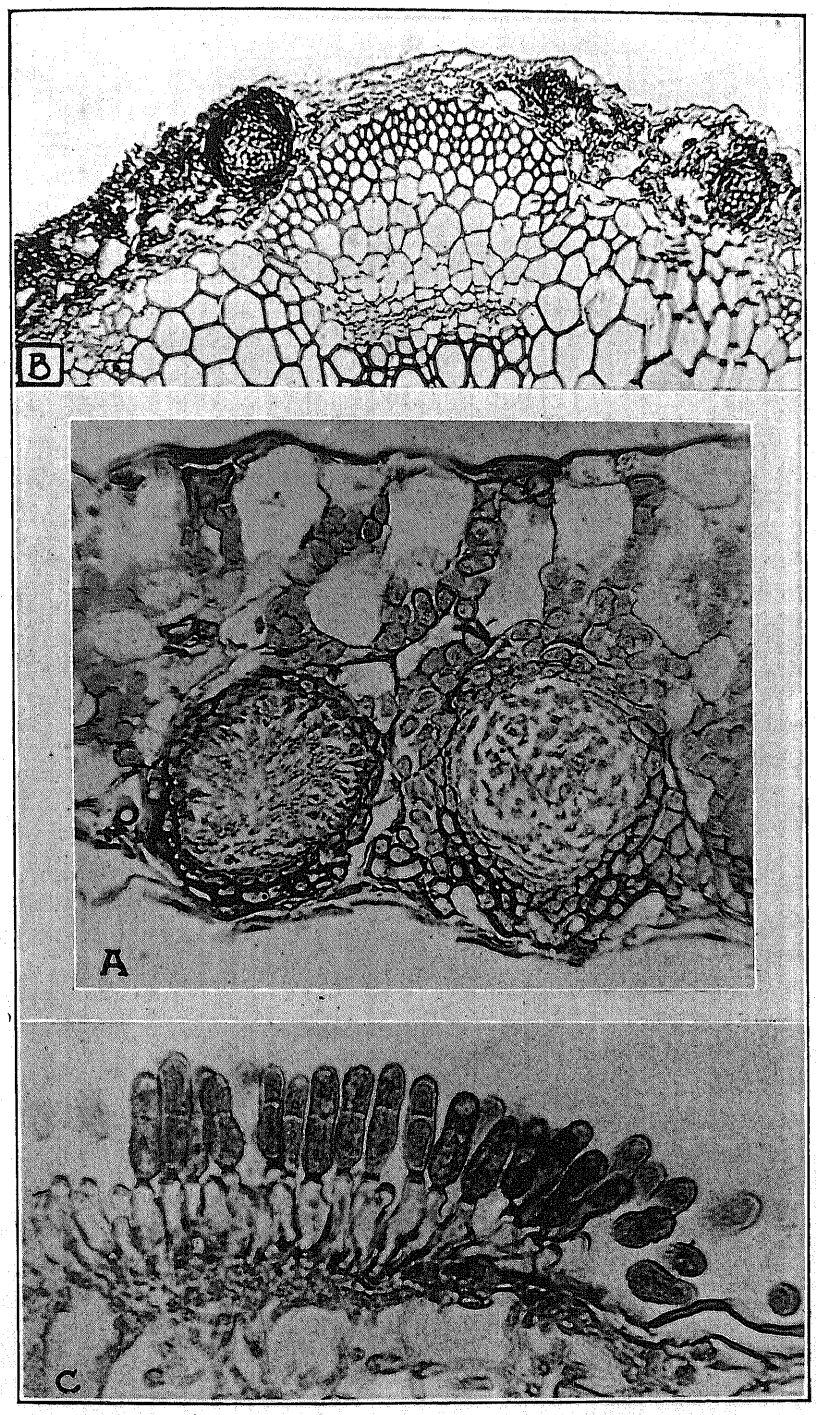
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PRELIMINARY REPORT OF SOME OBSERVATIONS ON ASCOSPORE DISCHARGE AND DISPERSAL OF CONIDIA OF VENTURIA INAEQUALIS (COOKE) WINTER

J. E. Howitt and W. G. Evans

The general life history of *Venturia inaequalis* (Cooke) Winter has been known for many years. The inoculum for primary infection in the spring of the year comes chiefly in the form of ascospores from the fallen leaves. Secondary cycles are initiated by conidia produced in the developing scab spots. Spore discharge of the apple scab fungus has received considerable attention from plant pathologists. Wallace (7), Childs (2), Curtis (3, 4), Keitt and Jones (5), Schneiderhan (6) and Adams (1) have all published important contributions to our knowledge on this subject. There are, however, many problems of importance in regard to the details of the life history which have not been completely solved. Some of these are: the relationship of temperature and precipitation to ascospore development and discharge; the length of period of ascospore vitality; and the dissemination of conidia through the orchard.

With the hope of throwing more light on these and other problems, observations have been made and recorded during the past six years. Unfortunately the records for 1921 have been mislaid and consequently cannot be included in this preliminary report. These observations have been made each year in a neglected orchard near the Ontario Agricultural College and supplemented by observations made in other neglected orchards in the neighborhood. Records have been kept of the date of first ascospore discharge for each year and the date of free ascospore discharge for each year. In addition the development of the apple buds at the time of ascospore discharge also was recorded. These observations were supplemented by germination tests and field experiments.

The time of first ascospore discharge and free ascospore discharge was determined by gathering apple leaves at different dates during the spring and examining the perithecia to ascertain the stage of development of the asci. The date of first ascospore discharge was judged by the first appearance of empty asci in the perithecia. The date of free ascospore discharge was considered to be the date when fifty per cent or more of the asci were found to have discharged their spores. The dates recorded therefore are necessarily but approximate. They should, however, suffice for a basis of comparison.

THE RELATIONSHIP OF TEMPERATURE AND PRECIPITATION TO ASCOSPORE DEVELOPMENT AND DISCHARGE

Such a relationship has long been recognized by all who have worked with apple scab. The consensus of opinion appears to have been that the amount of precipitation during April and May was the important factor influencing the development and discharge of the ascospores. The figures given in the table below, while not absolutely conclusive, strongly indicate that the temperature during the winter months may be a factor, the importance of which up to the present time has not been fully recognized.

Table 1 records figures for the years 1919, 1920, 1922, 1923 and 1924 only. The records for 1921 have been mislaid, and the records for 1925, not having been made in the neighborhood of Guelph, are not comparable. The available soil temperatures at the depth of one inch have also been included to show that the varying snowfalls of the different seasons did not appear to alter the ratio of the average mean temperature for the winter months of the years recorded.

A study of the figures in the table brings out the following points.

First, that apparently the average mean temperature of the months of January, February and March has more influence upon ascospore development and primary discharge of ascospores in the spring than has the amount of precipitation from April 1 to primary ascospore discharge. These figures do not prove this conclusively but they at least strongly suggest it.

Second, that the average mean temperature for January, February and March is more closely related to ascospore development and discharge than is the mean temperature of the days in April and May preceding primary ascospore discharge.

When the average mean temperatures for the preceding six months, namely, October, November, December, January, February and March, of the various years recorded were compared with the dates of primary ascospore discharge and free ascospore discharge, a correlation was found which, although not complete, was sufficiently marked to indicate that the temperatures of the preceding six months, as well as those of January, February and March only, may have an influence upon ascospore development and discharge.

LENGTH OF PERIOD OF ASCOSPORE VITALITY

The length of time that ascospores capable of germination remain in the orchard is also a question of some importance. Observations throwing some light on this point were made in 1925. The first mature ascospores were found on April 23. Each week from this date until August 8, viable ascospores were found. The vitality of the ascospores was determined by germination tests made by placing the ascospores in water on slides. These tests were made at ordinary room temperatures. It is interesting to note

TABLE 1.—*Precipitation and temperature in relation to ascospore development and discharge in Venturia inaequalis*

Year	1919	1920	1922	1923	1924
Date of first observed ascospore discharge	April 28	May 17		May 18	May 9
Date of free ascospore discharge	May 6	May 31	May 10	May 26	May 17
No. days from April 1 to free ascospore discharge	36	61	40	56	47
No. days from first observed ascospore discharge to free ascospore discharge	8	14		8	8
No. days of precipitation from April 1 to free ascospore discharge	15	26	8	22	20
Amount of precipitation in inches from April 1 to first ascospore discharge	2.58	3.69		5.61	4.64
Amount of precipitation in inches from first ascospore discharge to free ascospore discharge	3.04	.65		1.43	3.09
Mean temperature, January	26.3	9.59	18.97	18.8	19.4
Mean temperature, February	25.3	14.55	23.18	14.1	16.33
Mean temperature, March	24	30.67	32.08	24.28	27.9
Average mean temperature for January, February, and March	28.53	18.27	28.07	19.06	21.21
Average mean soil temperature at depth of 1 inch for January, February, and March	30.23	27.39	29.65		
Mean temperature from April 1 to first ascospore discharge	39.2	42.5		43.5	42.5
Mean temperature from April 1 to free ascospore discharge	40.4	45.36		43.5	44.5
Mean temperature from first ascospore discharge to free ascospore discharge	44.8	60.1		53.3	50.2

that apple leaves collected on August 7 contained perithecia filled with immature asci in which the protoplasm was still undifferentiated, and that apple leaves which had been kept in a cage outside under some trees, where they were exposed to temperature changes but not to soaking by rain, were found to contain asci and ascospores when examined in December.

DISSEMINATION OF CONIDIA THROUGH THE ORCHARD

The question as to whether conidia are blown by the wind from tree to tree in an orchard is one of some practical importance but one upon which there appears to be little evidence based on experiments. Some pathologists evidently assume that such dissemination does take place, while others appear to think that there is little danger of conidia blowing from tree to tree. To secure evidence bearing on this question, experiments were conducted in 1925.

On June 5, a number of stakes were placed at an angle of 45° to the ground around an isolated tree in an orchard. These stakes were located at distances of from 3 to 40 feet from the tree affected with scab. Three to five microscopic slides, lightly covered with vaseline, were fastened to each of these stakes. Similar slides were also fastened to nearby fence posts. On June 30, a few conidia were found on each slide. These slides were on stakes placed 3, 6, 10 and 15 feet from the tree trunk. On July 2, a few conidia were found at 3 and 10 feet from the trunk. On July 8, conidia were found west and southwest of the tree on slides attached to stakes at distances of 5, 12, 15 and 25 feet from the trunk, and on slides on two fence posts 50 feet from the tree trunk. At this time scab was developing rapidly on the leaves of the tree. On July 16, another set of similar slides was placed around the tree. On July 20, slides at 10, 20, 30 and 40 feet north of the tree showed conidia; and on July 28, slides on stakes located 5, 10, 20, 30, 40 and 50 feet west of the tree showed conidia. Conidia were also found on slides fastened to fence posts 75 feet from the tree. This tree was nearer the slides than any of the other apple trees. The conidia collected on these slides were not distinguishable from the conidia scraped from the scab spots on the leaves. On slides placed in a field at some distance from the orchard, no spores resembling the conidia of *Venturia* were found.

SUMMARY

1. The mean temperature of the winter months, especially of January, February and March, has a marked influence upon ascospore development and primary discharge in the spring in *Venturia inequalis*.

2. Ascospores, in Ontario, may retain their vitality at least until the eighth of August, and probably until a much later date.

3. Conidia may be freely scattered by wind from tree to tree in an orchard.

ONTARIO AGRICULTURAL COLLEGE,
GUELPH, ONTARIO.

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SEED TREATMENT EXPERIMENTS FOR CONTROLLING STRIPE DISEASE OF BARLEY[1]

R. W. Leukel, James G. Dickson, and A. G. Johnson

INTRODUCTION

During the past fifteen years, various workers have conducted numerous experiments to find a satisfactory seed treatment for the control of stripe disease of barley caused by *Helminthosporium gramineum* Rabh. Most of this work was done in Europe, where stripe disease appears to be most serious. The earlier experiments were confined mostly to the use of hot water, mercuric chloride, copper sulphate and formaldehyde, but organic mercury and other compounds were tried as early as 1914.

Mortensen (17) in 1909 tried the hot-water treatment. Störmer (34) in 1912 also tried hot water as well as copper sulphate and formaldehyde. Appel and Riehm (4) in 1913 tried hot water with varying results. Johnson (11) in 1914 reported good results from the use of hot water and formaldehyde. Spiekermann (33) in 1914 failed to control stripe by the use of hot water and formaldehyde. Riehm (27) in 1914 and again (28) in 1915 reported excellent results from the use of chlorophenol mercury. Johnson (12) in 1916 used formaldehyde and copper sulphate with good results. Kiessling (14) in 1916 reported partial control with Uspulun but found considerable variation in its effectiveness on different varieties.

Müller and Molz (18) in 1918 experimented with a large number of substances not commonly used for seed treatments, their aim being to eliminate bird and rodent depredations, as well as stripe disease. Lind and Ravn (16) in 1918 reported the results of eight years' work with various seed treatments and recommended copper sulphate, mercuric chloride or formaldehyde for severely infected seed, and hot water for slightly infected seed. Experiments in Scotland (1) in 1919 included these same treatments. Köck (15) in 1919 recommended formaldehyde and Uspulun. Müller, Molz and Shröder (19) in 1920, reporting the results of three years' experiments, stated that Germisan controlled stripe disease perfectly, while Uspulun and Corbin reduced the percentage of infection from 30 per cent to 4.7 and 6.3 per cent, respectively.

Riehm (29) in 1921 reported good results with Uspulun and Fusafine. Nolte and Gehring (22) in 1921 reported excellent results from the use of

[1] Investigations conducted cooperatively between the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

formaldehyde, Uspulun and Fusafine. Müller and Molz (20) in 1921 again reported complete control of stripe disease with Germisan, and fair control with Uspulun. Fraser (8) in 1921 reported satisfactory control with hot water and formaldehyde, reducing the percentage of stripe disease from about 60 per cent to 11 per cent with the former, and to 5 per cent with the latter. Verhoeven (35) in 1921 secured approximate control by spraying different lots of seed with solutions of copper sulphate, Germisan 14 B, and Uspulun. He tried soaking the seed in Uspulun solution for 1½ hours and then in hot water (52° C.) for 10 minutes. He also used a hot Uspulun solution after previously soaking the seed in cold water. In both cases, he reported excellent stripe control.

Riehm (30) in 1921 recommended formaldehyde and Uspulun for perfect control of stripe, and stated that mercuric chloride, copper sulphate and Fusafine greatly reduced the amount of infection. Schaffnit (32) in 1922 reported complete control of stripe disease with Germisan, and approximate control with Uspulun. He found these two the only effective treatments among a considerable number tried. In 1923, van Poeteren (25) recommended Uspulun, Germisan and copper sulphate. Ausborn (5) in 1923 recommended Germisan, Uspulun and hot water. Verhoeven (36) in 1923 stated Germisan produced the best results in stripe control, while Uspulun was fairly good. Dreger (6) in 1923 secured perfect control by using Uspulun after hot water. Remy and Vasters (26) in 1923, after reviewing the work done on seed treatment from 1913 to 1920, recommended chlorophenol mercury for perfect control of stripe disease. Müller, Molz and Müller (21) in 1923 again recommended Germisan and Uspulun.

Plaut (23) in 1924 reported favorable results with Germisan, Uspulun and Tillantin "C," and again (24) in 1925 with the first two. He also tried many other treatments, including hot solutions of some chemicals. Adams (3) in 1924 advised treatment with Semesan. Ferdinandsen (7) in 1924 pronounced Germisan the best preparation yet discovered for the control of stripe disease.

Gram (9) in 1924 recommended Germisan as being superior to Uspulun and copper sulphate for stripe control. In another report (2) in 1924 Germisan, Tillantin "C" and "Hohenheimer Beize" are recommended. Johnson, Leukel and Dickson (13) in 1924 reported perfect control of stripe disease with solutions of Semesan, Chlorophol, Germisan, Corona 610 and Corona 620, without seed injury. Gram (10), in 1925, after extensive experiments covering seven years, recommended Germisan above all other treatments, and next to that, Tillantin "C." Rodenhiser and Stakman (31) in 1925 reported satisfactory control of stripe disease with hot solutions (52° C.) of Germisan, Uspulun and Semesan. Germisan solution at room temperature also was satisfactory.

The foregoing summary of the more pertinent literature on seed treatments for the control of stripe disease of barley shows considerable variation in the results obtained by different workers using seed-treatment materials of the same name. Following are some of the probable reasons for these apparent discrepancies.

1. The variety of seed used. Some varieties respond to treatment more readily than others (4, 15).
2. The severity of the seed infection.
3. Differences in the methods and the details of applying the treatments, as, the temperature of the solution, the amount of stirring and mixing, the degree of contact between seed and solution, the duration of the treatment, the method of drying the seed, and the number of times the solution was used without renewal.
4. Variation in the chemical composition of compounds of the same name used at different times or by different workers.
5. Variation in the conditions obtaining at the time of sowing the seed, such as soil temperature, soil moisture and soil reaction.
6. Lack of adequate planting replications.
7. A difference of opinion as to what constitutes "satisfactory control."

The inoculum furnished by a small percentage of stripe disease in a crop may cause considerable seed infection. It seems that unless seed treatment is to be practised every year, a satisfactory treatment for stripe disease should eliminate the disease completely without seed injury.

In earlier experiments conducted by the writers, hot water, formaldehyde and copper sulphate were not found entirely satisfactory, chiefly because of seed injury and lack of consistent effectiveness.

In the fall of 1922, the writers started experiments with a number of the most promising, new seed-treatment materials. A summary of the earlier results has been published (13). The manufacture of some of these compounds first used has been discontinued, while other compounds have been discarded because of ineffectiveness. On the other hand, new seed-treatment compounds appeared upon the market too late to be included in the experiments here described.

MATERIALS

In the spring of 1925, rather extensive seed-treatment experiments were undertaken, especially with liquid treatments. Only those compounds that seemed most promising and were then available were used. All compounds employed were of recent manufacture. The following compounds were used:

A. For liquid treatments.

1. Uspulun, a hydroxymercurichlorophenol sulphate preparation, produced by the Bayer Company, Inc., New York City.
2. Bayer Compound, a hydroxymercurinitrophenol sulphate compound, also produced by the Bayer Co., Inc., New York City.
3. Germisan, a mercury cresol-sodium cyanide compound, manufactured by the Saccharin-Fabrik Aktiengesellschaft, vorm Fahlberg List and Company, Magdeburg, Germany.
4. S. F. A. No. 125, and
5. S. F. A. No. 175; the chemical composition not known to the writers; both manufactured by the Saccharin-Fabrik Aktiengesellschaft, vorm Fahlberg List and Company, Magdeburg, Südost, Germany.
6. Semesan, a chlorophenol mercury compound made by E. I. du Pont de Nemours and Company, Inc., Wilmington, Del.
7. Corona No. 620, a mercurated ortho-nitro-phenol and
8. Corona No. 640, a mercurated acetaldehyde; manufactured by the Corona Chemical Division of the Pittsburgh Plate Glass Co., Milwaukee, Wis.
9. Tillantin "C," a colloidal copper and organic arsenic compound made in Germany and sold in this country by H. A. Metz and Company, Inc., 122 Hudson St., New York City.

B. For dust treatments.

10. Uspulun-Bolus, similar in composition to Uspulun, but in the form of a dust, and
11. Bayer No. 3, the same as Bayer Compound, but to be used in dust form; manufactured by the Bayer Company, Inc., New York City.
12. S. F. A. No. 225, manufactured by the makers of Germisan.
13. Semesan, the same as No. 6, used as a dust.
14. Semesan Jr., made by the manufacturers of Semesan.
15. Copper Stearate, made by the manufacturers of Corona No. 620.

The seed used in the 1925 experiments was Oderbrucker barley, Wisconsin Pedigree No. 6 (C. I. No. 1146), secured from the Agronomy Department of the Wisconsin Agricultural Experiment Station and grown on West Hill Experiment Farm near Madison, Wis. Three lots of this seed from the 1922, 1923, and the 1924 crops, respectively, were used for the liquid treatments. The seed had been cleaned by means of a fanning mill before it was received.

All the seed treatments were applied at Arlington Experiment Farm, Va., by one of the writers, in February, 1925.

METHODS AND RESULTS

Liquid Treatments. For the liquid treatments, 1, 0.5, and 0.25 per cent water solutions of each compound were carefully made up in glazed, earthenware jars. The temperature of the solutions was kept at about 18° C. throughout the treatments. The seed was placed in properly labeled, clean, cheesecloth bags, 100 to 200 gms. in each, and immersed in the solutions for the given lengths of time. After being allowed to drain a few minutes in the bags, the seed was spread out on paper towels to dry. The drying was hastened by the use of electric fans. When thoroughly dry, the seed was packeted in envelopes, 16½ gms. in each, for subsequent sowings in rod-rows at the rate of one gram per linear foot of row.

On account of the shortage of the stripe-infected seed, a closely similar barley, Wisconsin Pedigree No. 9, 1924 crop, was used to test the effects of the treatments on germination. A portion of this seed was treated similarly and 500 kernels from each lot of treated seed and eight lots of 500 kernels each of untreated seed were sown in the field at Arlington Experiment Farm, Va., April 13, 1925. The germination counts, made May 2, are shown in table 1.

TABLE 1.—*Germination percentages of Wisconsin Pedigree No. 9 barley, grown in 1924, untreated or treated with different seed disinfectants and sown in the field in paired rows, 250 seeds per row, replicated twice, at Arlington Experiment Farm, April 13; data recorded May 2, 1925*

Seed disinfectants	Germination percentages of seed						
	Soaked 1 hour in water solutions of:			Soaked 2 hours in water solutions of:			Untreated
	1 per cent	0.5 per cent	0.25 per cent	1 per cent	0.5 per cent	0.25 per cent	
Uspulun	92	94	92	89	91	91	77
Bayer Compound	82	90	91	81	86	86	79
Germisan	78	82	85	73	85	83	79
S.F.A. No. 125	80	86	77	79	91	94	82
S.F.A. No. 175	83	91	91	68	84	90	83
Semesan	91	90	86	92	92	86	81
Corona No. 620	55	67	75	40	57	70	77
Tillantin "C"	73	79	79	50	78	76	77

The field sowings of the treated and the untreated stripe-infected seed were made in three essentially parallel series, as follows:

Series I at Arlington Farm, Va., March 16, 1925, Series II and III at Madison, Wis., April 11 and May 9, 1925, respectively.

TABLE 2.—*Summary of data on stripe-disease control in Wisconsin Pedigree No. 6 barley, seed untreated or treated with different seed disinfectants at Arlington Experiment Farm, Va., and sown as follows:*

Series I at Arlington Farm, Va., March 16, 1925. Six replications.
Series II at Madison, Wis., April 11, 1925. Five replications.
Series III at Madison, Wis., May 9, 1925. Four replications.

Seed disinfectants	Seed from crop of	Total number of stripe-infected plants from seed soaked for one hour in a water solution of											
		1 per cent				0.5 per cent				0.25 per cent			
		Series I	Series II	Series III	Total	Series I	Series II	Series III	Total	Series I	Series II	Series III	Total
Uspulun	1922	0	1	1	2	0	2	0	2	2	2	1	5
	1923	0	0	.....	0	0	0	.....	0	2	4	.....	6
	1924	0	0	0	0	4	0	0	4	3	2	0	5
	Total	0	1	1	2	4	2	0	6	7	8	1	16
Bayer Compound	1922	0	0	0	0	0	1	1	2	1	0	2	3
	1923	0	0	.....	0	0	1	.....	1	0	4	.....	4
	1924	4	1	0	5	8	1	0	9	15	6	4	25
	Total	4	1	0	5	8	3	1	12	16	10	6	32
Germisan	1922	0	0	0	0	0	0	0	0	0	0	0	0
	1923	0	0	.....	0	0	0	.....	0	0	0	.....	0
	1924	0	0	0	0	0	0	0	0	0	0	0	0
	Total	0	0	0	0	0	0	0	0	0	0	0	0
S.F.A. No. 125	1922	0	0	0	0	2	3	0	5	16	9	1	26
	1923	0	2	.....	2	2	2	.....	4	5	5	.....	10
	1924	7	4	0	11	14	11	0	25	27	23	3	53
	Total	7	6	0	13	18	16	0	34	48	37	4	89
S.F.A. No. 175	1922	0	1	0	1	0	2	0	2	20	4	0	24
	1923	1	0	.....	1	0	1	.....	1	5	7	.....	12
	1924	1	2	0	3	7	2	0	9	24	14	1	39
	Total	2	3	0	5	7	5	0	12	49	25	1	75
Semesan	1922	1	1	1	3	7	2	0	9	14	9	3	26
	1923	0	1	.....	1	0	0	.....	0	6	1	.....	7
	1924	4	3	0	7	9	1	2	12	21	8	1	30
	Total	5	5	1	11	16	3	2	21	41	18	4	63
Corona No. 640	1922	98	47	13	158	128	76	22	226	150	76	21	247
	1923	29	19	.....	48	50	33	.....	83	71	41	.....	112
	1923	89	41	16	146	99	62	16	177	107	76	21	204
	Total	216	107	29	352	277	171	38	486	328	193	42	563
Corona No. 620	1922	1	0	0	1	4	0	0	4	2	1	0	3
	1923	0	0	.....	0	1	0	.....	1	0	0	.....	0
	1924	0	0	0	0	4	1	0	5	5	0	0	5
	Total	1	0	0	1	9	1	0	10	7	1	0	8
Tillantin "C"[a]	1922	1	0	0	1	0	0	0	0	0	1	0	1
	1923	0	0	.....	0	0	0	.....	0	1	0	.....	1
	1924	.....	0	0	0	.....	0	0	0	.....	0	0	0
	Total	1	0	0	1	0	0	0	0	1	1	0	2

[a] Only three replications in Series I.

Total number of stripe-infected plants from seed soaked for two hours in a water solution of												Number of stripe-infected plants from untreated seed				
1 per cent				0.5 per cent				0.25 per cent								
Series				Series				Series				Series				
I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total	Average per row
0	0	0	0	1	1	0	2	0	3	0	3	139	96	20	255	21
0	0	.....	0	0	0	.....	0	0	1	.....	1	117	77	.....	194	32
0	0	1	1	3	0	2	5	1	1	1	3	127	95	24	246	21
0	0	1	1	4	1	2	7	1	5	1	7	383	268	44	695	.....
0	0	0	0	0	0	0	0	0	3	1	4	.....	97	37	134	17
0	0	.....	0	0	1	.....	1	1	0	.....	1	.....	69	.....	69	17
3	1	2	6	4	3	0	7	13	2	2	17	.....	103	22	125	16
3	1	2	6	4	4	0	8	14	5	3	22	.....	269	59	328	.....
0	0	0	0	0	0	0	0	0	0	0	0	166	107	33	306	26
0	0	.....	0	0	0	.....	0	0	0	.....	0	130	55	.....	185	31
0	0	0	0	0	0	0	0	0	0	0	0	119	98	22	239	20
0	0	0	0	0	0	0	0	0	0	0	0	415	260	55	730	.....
2	0	0	2	6	1	0	7	11	6	2	19	.....	108	27	135	17
0	0	.....	0	1	1	.....	2	3	2	.....	5	.....	75	.....	75	20
0	0	0	0	9	3	0	12	19	7	2	28	.....	77	18	95	12
2	0	0	2	16	5	0	21	33	15	4	52	.....	260	45	305	.....
0	0	0	0	0	0	0	0	3	1	1	5	142	93	24	259	22
0	0	.....	0	0	0	.....	0	4	1	.....	5	123	55	.....	178	29
2	0	0	2	3	5	0	8	13	5	3	21	188	89	22	299	25
2	0	0	2	3	5	0	8	20	7	4	31	453	237	46	736	.....
0	1	0	1	5	0	2	7	15	2	4	21	.....	97	32	129	16
0	0	.....	0	0	0	.....	0	1	2	.....	3	.....	49	.....	49	12
1	0	0	1	4	5	1	10	14	0	6	20	.....	94	10	104	13
1	1	0	2	9	5	3	17	30	4	10	44	.....	240	42	282	.....
85	54	18	157	150	67	19	236	198	84	27	309	212	87	40	339	27
27	10	.....	37	55	27	.....	82	83	33	.....	116	109	49	.....	158	26
64	37	10	111	96	68	13	177	114	64	13	201	149	97	29	275	23
176	101	28	305	301	162	32	495	395	181	40	626	470	233	69	772	.....
0	1	0	1	2	2	0	4	2	1	0	3	.....	122	31	153	19
0	0	.....	0	1	0	.....	1	0	0	.....	0	.....	56	.....	56	14
0	0	0	0	1	0	2	3	1	1	0	2	.....	93	17	110	14
0	1	0	1	4	2	2	8	3	2	0	5	.....	271	48	319	.....
0	0	0	0	0	0	0	0	0	0	0	0	230	126	32	388	32
0	0	.....	0	0	0	.....	0	1	0	.....	1	108	56	.....	164	29
.....	0	0	0	.....	0	0	0	.....	1	0	1	.....	100	26	126	16
0	0	0	0	0	0	0	0	1	1	0	2	338	282	58	678	.....

Unfortunately, the limited amount of 1923 seed available made it necessary to sow three replications less of this seed lot than of the 1922 and 1924 seed lots of which six replications were sown for each treatment, with the exception of Tillantin "C." A slight shortage of 1924 seed made it necessary to omit the treatment of this seed lot with Tillantin "C," Series I.

Infection counts on the first, second and third series were made May 20–22, July 2–6 and July 15–17, respectively, the number of diseased plants per row being recorded. Representative rows from each treatment and from the controls were pulled and the plants counted so that the number of striped plants per row could be expressed in terms of approximate percentages.

The results showing the degree of stripe control by each of the liquid treatments in all three series are summarized in table 2.

Yields were obtained from certain representative rows in the second series when the grain had ripened. For this purpose rows were selected in which the stripe control was best, together with nearby control rows. These rows were carefully harvested and threshed separately. The threshing was done at the West Hill Experiment Farm, Madison, Wis., with the rod-row thresher of the Agronomy Department, Wisconsin Agricultural Experiment Station. Immediately after threshing, the yield from each row threshed was cleaned in a small fanning mill. The cleaned grain was then carefully weighed. The yields are given in table 3.

Dust Treatments. Preliminary experiments with dust treatments including copper carbonate, Seed-o-san, Corona 640, Napthal dust and a mixture of Semesan and Lux failed to disclose any that satisfactorily eliminated stripe disease.

Another series of dust treatment experiments of limited scope was carried out at Arlington Experiment Farm, Va., in the spring of 1925. Seed from the crops of 1922 and 1923 was used. The dusts were applied by placing sixty grams of the infected seed in a large-mouthed bottle and adding a heaping teaspoonful of the dust to be tested. The bottle was corked and shaken thoroughly until the seeds were coated as much as possible. The excess dust was removed by pouring the seed on a coarse sieve. A different bottle was used for each treatment. The seed from each treatment was sown at Arlington Experiment Farm, Va., March 21, 1925, in paired rod-rows replicated twice. Untreated controls were sown at the same time. The same rate of seeding was used as in the case of the liquid treatments, and the germination percentages of the treated and the untreated seed also were determined in the same manner. Infection counts were made May 27. The germination and infection data are shown in table 4.

TABLE 3.—*Rod-row yields of threshed barley grown from treated seed and from untreated seed in adjacent rod-rows, the average yield per row, and the average approximate percentage of striped plants in each row.*

Replication number	Yields in grams from seed treated with compounds indicated or untreated											
	Uspulun[a]	Untreated	Bayer Comp.[a]	Untreated	Germisan[b]	Untreated	Semesan[a]	Untreated	Corona 620[b]	Untreated	Tillantin "C"[b]	Untreated
1	662	524	590	484	420	484	330	290	350	282	326	282
2	662	520	550	464	420	464	310	260	340	304	310	304
3	612	412	374	390	416	390	500	360	458	286	418	286
4	560	454	420	462	464	462	454	362	442	400	422	400
5	560	508	652	490	632	444	470	444	512	532	600	532
6	494	490	624	508	622	566	452	404	540	472	636	472
7	680	670	544	574	503	600	552	540	490	374	436	374
8	660	574	508	670	690	654	590	640	434	324	514	324
Total	4890	4152	4262	4042	4167	4064	3658	3300	3566	2974	3662	2974
Average	611	519	533	505	521	508	457	413	446	372	458	372
Average percentage stripe	t[c]	8	t	8	0	7	t	7	0	9	t	9

[a] Seed soaked one hour in 0.5 per cent solution.

[b] Seed soaked one hour in 0.25 per cent solution.

[c] t = trace.

TABLE 4.—*Stripe disease control in Wisconsin Pedigree No. 6 barley, seed untreated, or treated with different dust disinfectants and sown in rod-rows at Arlington Experiment Farm, Va., March 21, 1925. Infection count—May 27, 1925*

Treatment	Number of striped plants per rod-row from				Total	Average	Approximate per cent infection	Per cent germination
	1922 seed		1923 seed					
	Row 1	Row 2	Row 3	Row 4				
Check	50	69	55	50	224	56	17	83
Uspulun	45	40	36	38	159	40	12	82
Bayer No. 3	26	27	18	15	86	22	7	85
Check	80	65	61	65	271	68	21	82
S.F.A. No. 225	2	2	0	0	4	1	0.3	87
Semesan Jr.	8	4	1	6	19	5	2	83
Check	65	55	80	60	260	65	20	81
Semesan	27	25	0	0	52	13	4	88
Copper stearate	50	55	60	55	220	55	17	86
Check	60	65	39	40	260	65	20	82

SUMMARY

1. From the standpoint of effects on germination it will be noted from table 1 that Corona 620 produced the greatest reduction in the percentage of germination compared with the untreated controls, although S.F.A. No. 175 and Tillantin "C" at 1 per cent for two hours also caused marked injury to germination. The other treatments either did not affect germination or apparently improved it to some extent. Uspulun-treated seed showed the highest average percentage of germination throughout, while next in order came seed treated with Semesan, Bayer Compound, S.F.A. No. 125 and No. 175, Germisan, Tillantin "C" and Corona 620.

2. The Germisan treatments were the only ones that completely eliminated stripe disease, although some of the others reduced it to a very slight trace. Therefore, if stripe-free seed is the aim of the grower, it seems that treatment with Germisan can be recommended, whereas if the only aim of the grower is to reduce the loss due to stripe in the crop grown from badly infected seed, or if seed treatment is to be practised every year, a number of the other disinfectants will serve the purpose equally well. It will be noted that while treatment with Uspulun resulted in the highest percentage of germination (table 1), it also resulted in the highest yield (table 3) while it reduced the stripe disease to a slight trace (table 2). Germisan, on the other hand, while controlling stripe disease perfectly, did not improve the germination of the seed nor materially increase the yield of grain. Semesan, also a chlorophenol mercury compound like Uspulun, was almost equal to Uspulun in its effect on percentage of germination, stripe control, and yield of grain. The same is true of Bayer Compound.

3. According to the data presented, it seems that either Germisan or Uspulun produced the best results, depending on the purpose of the treatment. At any rate, several of the treatments were superior to formaldehyde and copper-sulphate treatments which invariably have been found to produce seed injury.

4. The data presented in table 4 show that the dusts had no apparent effect on the germination of the seed, the treated and untreated seed showing about the same percentage of germination. All of the dusts decreased the amount of stripe to some extent, and a few almost eliminated it. The effectiveness of dust treatments for stripe disease probably depends to a great extent on the amount of moisture in the soil at the time of sowing the seed. Therefore, more work on the relation of soil moisture to the effectiveness of dust treatments is desirable.

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AN IMPROVED METHOD OF ISOLATING PSEUDOMONAS TUMEFACIENS SM. AND TOWN.

M. K. PATEL

In connection with our cultural and isolation studies of the organism causing crown gall (*Pseudomonas tumefaciens* Sm. and Town.), it was found that a small amount of sodium taurocholate in dextrose agar inhibits the development of most of the contaminating organisms and seemingly stimulates the growth of *Ps. tumefaciens*. The medium is made as follows: dissolve 15 grams of powdered agar in a double boiler, adding 3 grams of sodium taurocholate and 10 grams of Witte's peptone. After thoroughly digesting these two ingredients, add 20 grams of dextrose and lastly two cc. of a 1–1,000 aqueous solution of crystal violet. Filter twice through cotton and sterilize at 15 pounds pressure for 15 minutes. It is preferable to allow the freshly sterilized agar to cool to 40° C. and to pour the plates immediately, rather than to allow the agar to solidify and then remelt.

The utilization of this medium makes it possible to take large amounts of tissue with comparatively little dilution. Two or three pieces of tissue 10 x 10 x 2 mm. from different parts of the gall are finely chopped in 5 cc. of sterile distilled water in a sterile petri dish. The macerated material is allowed to stand for two to twelve hours to allow the bacteria to diffuse out of the tissue. One to two cubic centimeters of this suspension are placed with a sterile pipette into 50 cc. of melted bile agar, shaken well and used to pour five plates. The poured plates are incubated at 27°–30° C.

The advantages of this method are that less drastic disinfection of the gall tissue is required and a greater quantity of inoculum may be used; the development of the coccus forms and psychrophilic soil bacteria is inhibited by the bile and high temperature of incubation; the growth of the Gram-positive bacteria is inhibited by the crystal violet [Hexamethyl violet (chloride)].

This method does not exclude certain bacterial colonies which have at first a close resemblance to *Ps. tumefaciens* but which later seem to be quite distinct from those of the crown gall organism. *Ps. tumefaciens* is not inhibited, but develops as rapidly as on potato dextrose agar in pure culture.

By this method it is possible to recover *Ps. tumefaciens* from gall tissue, such as apple, much more readily than by using potato dextrose agar and the small amount of inoculum usually employed in dilution plates.

IOWA AGRICULTURAL EXPERIMENT STATION,
AMES, IOWA

PHYTOPATHOLOGICAL NOTES

An Improved Method for Isolation of Thielavia basicola. Recently being desirous of isolating the fungus *Thielavia basicola* in pure culture from tobacco roots affected with the Thielavia root rot and remembering the difficulty previously experienced in doing this by the poured plate methods usually employed in such cases and the similar experiences of others who had worked with this fungus, it occurred to me that tobacco seedlings might advantageously be used to assist in securing the pure culture. Accordingly seed of White Burley tobacco was sown quite thickly on blotting paper moistened with sterile water in small glass moist chambers. At the same time tiny bits of the dark brown, infected portions of the diseased tobacco roots above-mentioned were scraped off and scattered among the tobacco seed. The moist chambers were kept at room temperature, and in a few days the tobacco seed germinated. After about 10 days, when the seedlings were ¼ to ⅜ inch tall, it was noted that one had shrivelled at the base and fallen over. On microscopic examination it was found that the root and stem to the very leaves were parasitized by the mycelium of *Thielavia basicola,* and an abundance of spores, both endoconidia and chlamydospores, the former in greater number, were present on the seedling. During the next few days several of these parasitized tobacco seedlings were removed with sterile forceps from the moist chambers and laid on carrot agar plates, and in every instance pure cultures of the fungus were readily secured. The fungus grew out on the agar in most instances without the bacterial contamination usually encountered in making plates direct. At the same time several attempts made by other workers to isolate the fungus from the same material by the usual agar plate method either were unsuccessful or succeeded only after considerable difficulty and delay. The method is so simple, yet so successful, that it may be of assistance to others who have occasion to isolate *Thielavia,* or it may be adapted for use in other, similar cases.—W. W. Gilbert, U. S. Department of Agriculture, Washington, D. C.

Stain Technology. Under the auspices of the Commission on Standardization of Biological Stains, a journal, Stain Technology, has been started under the editorship of H. J. Conn, chairman of the commission, who represents the field of bacteriology. The fields of chemistry, zoology, pathology, and botany are represented respectively by the following associate editors: W. C. Holmes, S. I. Kornhauser, F. B. Mallory and W. R. Taylor. The journal is devoted primarily to the nature and uses of biological stains, including, however, articles dealing with microscopic technic in general when

they have a close bearing on staining procedures. Brief notes of only one or two paragraphs dealing with new staining procedures are welcome.

Authors who have matter for publication along the lines indicated are invited to submit it to the editor at the address: Lock Box 299, Geneva, New York.—H. J. CONN, Chairman, Commission on Standardization of Biological Stains.

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INFLUENCE OF THE ENVIRONMENT ON POTATO MOSAIC SYMPTOMS

C. M. TOMPKINS[1]

INTRODUCTION

The mosaic disease of potatoes is one of the most common and widespread of potato diseases. Because of its economic phases it has become a matter of considerable importance to understand the relation of environmental conditions to the disease as bearing on field inspection, seed certification, seed selection, and potato indexing. Environmental studies are of considerable scientific interest as well, since the nature of the causal agency of the virus diseases to which potato mosaic belongs never has been definitely ascertained. It is now generally recognized that low temperatures are conducive to the development of symptoms of potato mosaic and that high temperatures mask the symptoms. No information exists, however, as to how long these higher temperatures must be maintained in normal daily fluctuations in order to bring about masking. The investigations reported in this paper deal mainly with this problem, together with a study of other environmental conditions which might conceivably affect the symptoms of potato mosaic.

REVIEW OF PREVIOUS WORK

Potato mosaic was not definitely recognized as such until about 1911 (34), but the disease undoubtedly existed many decades before this. Schacht (40), as early as 1856, and Kühn (23), in 1872, noted that weather conditions influenced the Kräuselkrankheit of potatoes. This disease may have been caused by potato mosaic or a combination of potato viruses. In recent years, since the definite recognition of mosaic as a single disease or a group of diseases on the potato, a number of observers have noted that environmental conditions influence the symptoms: Stakman and Tolaas

[1] The investigations herein reported were conducted in the greenhouses of the Department of Plant Pathology at the University of Wisconsin, during the period 1923 to 1925. The writer is especially indebted to Drs. L. R. Jones and James Johnson for helpful advice and suggestions. This work was supported in part by funds appropriated by the Colorado Agricultural Experiment Station.

(43), Freiberg (12), Melhus (27), Murphy (28, 29, 30, 31, 32, 33), Schultz et al (41), Folsom (10), Schultz and Folsom (42, 11), Cotton (3, 4), Dickson (5, 7), Edgerton and Tiebout (9), Orton (35), Quanjer (38), Ducomet (8), Link (24), MacMillan (25), and Hungerford and Raeder (16). With the exception of Freiberg, however, none of these writers made any serious attempt to determine experimentally just what environmental factor was concerned with the modification of symptoms, although temperature among other factors was generally believed to be responsible.

Johnson (18, 19) was the first to make a detailed study in an attempt to determine which of the environmental factors were responsible for masking and where the dividing line occurred which separated the symptoms of mosaic from masking. Early recognizing the need for more reliable data on air temperatures, he undertook a series of experiments in an attempt to determine the critical air temperature for potato mosaic. He designed and constructed three simultaneously operated constant air-control chambers having thermostatic control, refrigeration within the cooler chambers, an automatically controlled humidifying system for each chamber, and means for renewal and circulation of air within the chambers. A description of the chambers has been published, but, since that time, they have been modified and improved. Johnson's method consisted of placing young potato plants already showing mosaic symptoms in the air-control chambers and keeping them there continuously for one to two weeks at various temperatures. It was found that the disease could be inhibited at as low a temperature as 23° to 24° C. Below that point symptoms of mosaic were readily induced, while above it masking occurred. Above the critical temperature, new leaves appeared, free from symptoms, while the symptoms in the older leaves gradually became less distinct and finally disappeared.

Goss (13), and Peltier and Goss (36), working in Nebraska under controlled conditions comparable to those under which Johnson's experiments were conducted, substantiated the latter's results in general. Further studies on the effect of air temperature recently were reported by Goss and Peltier (14); and a preliminary note on the principal conclusions drawn in the present paper has already been published (45).

Light is the only other environmental factor which has received special experimental consideration. Schultz and Folsom (42), Ducomet (8), MacMillan (25), Link (24), and Goss (13) seemed to agree that more distinct symptoms occurred on shaded than on unshaded potato mosaic plants, although Goss and Peltier (14) later concluded that light intensity had no effect on potato mosaic symptoms.

Soil temperature and soil moisture were believed by many writers to be responsible in part for the variation in potato mosaic symptoms as evidenced

by the observations of Ducomet (8), Murphy (30, 31), Orton (35), and Goss (13). In general, these writers noted that low soil temperatures and high soil moisture were apparently more favorable for mosaic symptoms on the potato. Goss and Peltier (14) later concluded that these factors had no direct effect upon potato mosaic symptoms.

Ducomet (8) and Link (24) believed that atmospheric humidity had some influence on potato mosaic symptoms.

Some attempt has been made to modify the symptoms of mosaic by varying the soil nutrients, apparently without success, as reported by Schultz and Folsom (42); although Perret (37) and Butler (2) claim to have masked mosaic by heavy manuring.

DESCRIPTION OF THE TYPE OF POTATO MOSAIC USED

Considerable confusion exists at present in regard to the classification and description of potato mosaic. It was first described as a separate form of virus disease by Orton (34) in 1914, the description being based upon observations made in Germany in 1911. Soon thereafter Quanjer (38) distinguished between mosaic and the leaf-rolling type of degeneration. At present, according to published reports, there are ten or more kinds of degeneration diseases.

Several classifications have been offered in recent years as a means of distinguishing between the various degeneration diseases of the potato. In 1921–1923, Quanjer (39) listed eight different potato virus diseases occurring in Europe, three of which are distinct types of mosaic. In 1921, Murphy (29) named but one type of potato mosaic, later adding a supplement (30) in which he distinguishes between simple mosaic and crinkle. Schultz and Folsom (42) have named seven types of degeneration diseases of the potato, three of which are forms of mosaic.

On the basis of these descriptions it is very difficult, if not impossible, for the widely separated workers to use them reliably and to interpret results therefrom. For instance, Quanjer (39) was not working with mosaic on the Triumph variety in Holland, and, inasmuch as he did not personally see the type of mosaic used at Wisconsin, it would appear that he may have been in error in stating that Johnson (19) confused crinkle with mosaic in experiments made in 1921. On the other hand, the confusion is apparently simply a question of nomenclature and a matter of acceptance of classification. Whatever may be the final name applied to the disease that Johnson worked with, it was unquestionably one form of potato mosaic and the same type as that with which this investigation is concerned. The type of mosaic worked with by Johnson in no way corresponds with Quanjer's crinkle, as judged either by the description or by the plate.

The type of mosaic used in connection with the intermittent air temperature and other environmental experiments will be referred to as typical Wisconsin Triumph mosaic. According to Schultz, who has personally seen the type of mosaic used in my experiments and in those of Johnson's, the marked type of Triumph mosaic should be classified as rugose mosaic. The description of mottling and wrinkling by Schultz and Folsom (42) satisfactorily describes these effects on Wisconsin Triumph potatoes. In general, mosaic potato plants have a chlorotic color compared with healthy ones.

In the following experimental data the term masking is used frequently. By masking is meant primarily the absence of the symptoms of mosaic on plants or parts of plants which are affected with the disease following the required incubation period. Complete masking makes it impossible to distinguish between mosaic and healthy leaves from plants produced from previously tested tubers. It is not understood that this represents recovery in the true sense of the word, because the virus is still present in the leaves, as may be demonstrated by exposing the plants constantly to air temperatures favorable for the development of symptoms.

EXPERIMENTAL RESULTS

Influence of Air Temperature

The experimental results secured by Johnson (19), and corroborated by Goss (13), apparently established the critical temperature for masking of the typical Wisconsin Triumph potato mosaic at approximately 24° C. under a continuous exposure to this temperature. The value of these data was interpreted particularly in terms of weekly, monthly, or seasonal variations in temperature. Under practical cultural conditions, however, crops are ordinarily subjected to a wide daily fluctuation in temperature. The critical temperature lies at such a point that under average conditions it is easily conceivable that during half of the day conditions are favorable for the disease and during the remaining half favorable for masking. Under mountain conditions in Colorado, or under northern conditions in the Wisconsin potato districts, high temperatures at noon and relatively low temperatures at night are generally the rule rather than the exception. Under these conditions of daily fluctuation of temperatures, masking occurs, as is evidenced by personal observations and reports from the observers to whom reference has already been made. It was natural to suppose, therefore, that the length of actual exposure to temperatures above and below the critical temperature might be of considerable importance in the phenomenon of masking. The experimental results on temperature reported in this paper are primarily concerned with the effect of varying lengths of ex-

posure of mosaic potato plants to temperatures favorable and unfavorable for symptoms of the disease.

Experimental Equipment and Methods Used. The experiments were performed in the greenhouses at the University of Wisconsin, using the same constant air-control chambers used by Johnson (18). Three chambers were operated simultaneously at different temperatures. The thermometers in the chambers were frequently compared with standard thermometers. The air temperature in each of the chambers usually did not vary more than one degree Centigrade. The humidity in the air-control chambers was regulated so that the evaporating power of the air in each chamber, as determined by Livingston atmometers, was approximately the same. Since the experiments in the greenhouses and in the chambers were conducted during the short days of the winter months, the plants naturally did not have normal light. However, judging from the appearance of the plants, they did not suffer seriously from lack of light, and it is not believed that this factor influenced the results.

Plants were exposed in the chambers simultaneously at such a time during the day as might most closely approximate field conditions. For example, in giving a two-hour exposure, plants were placed in the chambers at 11:00 A. M. each day and removed at 1:00 P. M. After exposure in the air-control chambers, the plants were returned to a cool greenhouse in which the air temperature was especially favorable for mosaic symptoms (ranging from 12° to 16° C.), and in which light and humidity conditions were uniformly good. The plants were kept in the cool greenhouse for 22 hours and then again placed in the chambers.

In all of the experiments, with but few exceptions which are noted elsewhere, Triumph stock grown under Wisconsin conditions was used. In several experiments, the People's was used, a late variety belonging to the Pearl group as given in Stuart's classification (44). Its culture in the United States is confined almost exclusively to western Colorado.

All tubers were first indexed in 4-inch porous clay pots as a test for mosaic. It was comparatively easy to differentiate between healthy and mosaic plants very soon after the young sprouts had broken through the soil. The young plants were then transferred to 6-inch pots and observed for one to several days prior to starting an experiment. The plants usually averaged about one inch in height at the time an experiment began. All experiments covered a period of at least two weeks. Well-drained fertile soil was used. Sufficient water was added to the soil each day to keep the plants well supplied, but no attempt was made to measure and regulate the amount of moisture. The soil temperature in the pots was approximately equal to the air temperature of the chambers.

In general, three mosaic plants and one healthy plant were placed in each air-control chamber; while the controls, consisting of the same number of plants, were kept in the cool greenhouse continuously.

Greenhouse Experiments. Mosaic and healthy plants one inch high were exposed in the air-control chambers 9 hours daily, from 7:30 A. M. until 4:30 P. M., at temperatures of 15°, 24°, and 28° C. respectively. Between 4:30 P. M. and 7:30 the next morning, the plants were kept in a cool greenhouse. The plants were in the chambers, therefore, 9 hours out of every 24, and in a cool greenhouse the remaining 15 hours. Control plants, healthy and mosaic, remained in the cool greenhouse continuously. Masking of the symptoms at the two higher temperatures was obtained in 7 days, *i.e.*, new leaves emerging from the bud showed solid dark-green, flat foliage, indistinguishable from healthy foliage. The lower or older leaves still showed some mottling, but the wrinkling effect had disappeared. With intermittent exposures, just as in the case of constant exposures, masking occurs first and proceeds more rapidly in young tissues than in older leaves. At 15° C., mottling and wrinkling remained unchanged, but small necrotic areas developed in the diseased leaf tissue. Likewise diseased control plants remaining continuously in the cool greenhouse

TABLE 1.—*Average leaf area of mosaic plants exposed for two hours a day at different temperatures*

Temperature in degrees C.	Average leaf area in square inches
15	11.41
24	12.53
28	23.90
15 (Controls)	8.13

developed necrotic areas. Healthy plants used as controls at the higher temperatures showed slight chlorosis and dropping of some of the lower leaves, while at the lower temperature and in the cool greenhouse the controls retained their dark green foliage. (See table 2 and Plate XXVIII.)

After the intermittent exposures were discontinued, the plants were allowed to remain in the cool greenhouse continuously. Mottling again developed after 13 days on new leaves of mosaic plants which had been exposed at 24° C. Symptoms became visible after 18 days on mosaic plants which had been exposed at 28° C. Masking is therefore only temporary, and mosaic symptoms are again induced by exposure to low temperatures. It is not known whether the virus is present in the leaf in the same proportions during the period of masking as it is when the leaf is mottled. This probably could be determined only by a series of dilution inoculations to healthy potato plants.

Four additional experiments were conducted at the same temperatures with intermittent exposures of 2, 3, 4, and 5 hours respectively. Results obtained were confirmatory of the experiment described above; these are given in table 2 (Plate XXIX). Referring to table 1, it may be noted that almost three times as much leaf tissue developed in the mosaic plants at 28° C. as in the controls (Plate XXX).

In a second series of experiments, diseased and healthy plants were exposed in the chambers at higher temperatures, namely, 20°, 30°, and 40° C., while the length of exposure varied from 2 to 6 hours (table 3, Plate XXXI). At 40° C., exposure seemed to induce not only excessive

TABLE 2.—*Time required for masking of mosaic plants exposed for different lengths of time at different temperatures*

Temperature in degrees C.	No. of hours of exposure a day	No. hours in greenhouse at 15° C. a day	No. days required for masking
15	9	15	No masking
	5	19	do
	4	20	do
	3	21	do
	2	22	do
24	9	15	7
	5	19	25
	4	20	33
	3	21	38
	2	22	42
28	9	15	7
	5	19	15
	4	20	17
	3	21	20
	2	22	28
15 (Controls)	0	24	No masking

chlorophyl production but stunting of the plants as well.

Temperatures of 15°, 24°, and 28° C. were used in a series of experiments with the People's variety in which exposures were made in the chambers at night (Plate XXVIII). Table 4 shows the time required for masking when plants were exposed for different lengths of time at different temperatures.

Again referring to table 2, it was found that mosaic plants can be masked in 15 days with a 5-hour exposure at 28° C. and in 25 days at 24° C.

Further experiments were conducted to determine whether masking could proceed to completion if a continuous 5-hour intermittent exposure were broken up into five integral parts; exposing the plants for one hour in the chambers, followed by one hour at a low temperature outside the chambers, then placing the plants in the chambers again for another hour, and continuing the process until a total exposure of 5 hours had been given. Triumph stock from Florida was used. Temperatures in the chambers were held at 23°, 29°, and 35° C. Plants were placed in the chambers at 7:30 A. M., 9:30 A. M., 11:30 A. M., 1:30 P. M., and 3:30 P. M., being removed therefrom during the alternate hours to a cool greenhouse. After completion of the total 5-hour discontinuous exposure, the plants were kept the remaining number of hours in the cool greenhouse. Diseased plants at 29° and 35° C. were masked in 8 days. This indicated that masking could be secured with a discontinuous exposure.

TABLE 3.—*Time required for masking of mosaic plants exposed for different lengths of time at different high temperatures*

Temperature in degrees C.	No. hours of exposure a day	No. hours in greenhouse at 15° C. a day	No. days required for masking
20	6	18	No masking
	4	20	do
	2	22	do
30	6	18	6
	4	20	9
	2	22	13
40	6	18	4
	4	20	5
	2	22	7
15 (Controls)	0	24	No masking

It then became of some interest to know whether a continuous 5-hour intermittent exposure could produce masking more quickly than a 5-hour discontinuous exposure. Two series of pots, each series having three mosaic plants and one healthy plant for each temperature, were used. Temperatures of 23°, 29°, and 35° C. were again employed. With one series the plants were exposed in the chambers for 5 consecutive hours, from 9:30 A. M. to 2:30 P. M. each day. The plants in the second series were run on a discontinuous schedule, commencing at 7:30 A. M. with exposures in the chambers. Masking was secured in 7 days in plants given a con-

tinuous 5-hour intermittent exposure, while a 5-hour discontinuous exposure required 13 days to bring about masking at 35° C.

Effect of Date of Planting on the Symptoms at High Altitudes. According to MacMillan (25), the symptom of mottling does not appear in potatoes grown at an altitude of 8,000 to 8,500 feet above sea level.

Tuber indexed stock was shipped from Wisconsin to Colorado in the spring of 1924 and planted at an elevation of 8,000 feet near Eagle, Colorado. This work was conducted as an experiment to test the effect of high altitude on Wisconsin mosaic stock and to determine whether or not, under field conditions, masking could take place as a result of short intermittent exposures at air temperatures above 23° to 24° C. Three plantings were made, 10 days apart, commencing on May 20 and ending June 9. The average day and night air temperatures are given in table 5.

The lot planted earliest came through the soil showing typical mottling, although it was not of the same pronounced type as is encountered in in-

TABLE 4.—*Time required for masking of mosaic plants exposed at night for different lengths of time at different temperatures*

Temperature in degrees C.	No. hours of exposure a night	No. hours in greenhouse at 15° C. a day	No. days required for masking
15	15	9	No masking
	9	15	do
	3	21	do
24	15	9	8
	9	15	17
	3	21	21
28	15	9	8
	9	15	12
	3	21	15
15 (Controls)	0	24	No masking

dexing work under greenhouse conditions. Mottling and wrinkling of the leaves were plainly visible until July 2, at which time all symptoms disappeared. With the second planting, symptoms were visible until the same date, when they too disappeared. The third lot never showed any symptoms. When the sprouts came up, the air temperature was above that of the critical temperature for short periods each day from June 24 on, long enough to inhibit the development of symptoms from the time of emergence from the soil, regardless of the fact that at night the air temperature dropped as low as 0° C. This small amount of field evidence

indicates that mottling may appear quite markedly in the high altitudes in Triumph stock if air temperatures are below 23° to 24° C., while higher temperatures mask the symptoms. The conclusion seems justified, therefore, that altitude has little, if any, effect on Triumph mosaic as far as mottling is concerned.

Conclusions from Air Temperature Experiments. The experimental results presented above are believed to be sufficient to justify the conclusion that relatively short exposures to high temperatures are sufficient to mask potato mosaic. This is of practical significance, since considerable fluctuations in air temperature occur throughout the entire 24 hours of the day in nature. High temperatures, as they occur under field conditions, are of an intermittent character and may persist for one to several hours. Consequently masking is not induced by constant exposures in the field but rather by intermittent exposures.

With intermittent exposures the rate of masking was found to be dependent largely upon the duration of exposure, upon the air temperature above 23° to 24° C., and upon the age of the leaves. In general, the higher the air temperature the more rapidly masking took place, within certain limits; while the rate of masking was retarded as the critical temperature was approached. Masking was first obtained and proceeded more rapidly in new young tissues than in older leaves with short intermittent exposures. Johnson's results were similar with constant exposures. At 30° to 40° C., the higher temperatures seemed to induce masking of new and old tissues with equal rapidity; at least no marked differentiation could be made between the rate of masking of old versus new tissues.

It seemed at first that masking might be associated with increased chlorophyl production. This appeared to be true when mosaic plants were exposed to intermittent high air temperatures. However, with constant exposure at similar high air temperatures, the leaves, while masked, were lighter green in color, a condition which suggested that masking may occur without increase in chlorophyl content in the leaves. In the earlier experiments, newly masked leaves produced at 24° C. contained decidedly less chlorophyl than new leaves at 28° C. The chlorophyl content developed at air temperatures of 30° and 40° C. was distinctly different from that which obtained at 24° and 28° C. Intermittent exposures at night, in comparison with day exposures, resulted in masked tissue which was paler green in color than that of plants exposed in daylight.

The first effect on the new leaves as they developed, produced by intermittent exposures during the process of masking, was wrinkling. The process was gradual but of relatively short duration. Before this was entirely accomplished, mottling began to disappear from the lamina. In general, masking proceeded from the base of the leaf next to the petiole

upward toward the apex. Masking was never considered complete in new leaves until they appeared without signs of mottling and wrinkling.

After masking, plants were then subjected to constant air temperatures below the critical air temperature, with the result that symptoms of mosaic reappeared. A longer period elapsed before the symptoms returned after a 6-hour exposure than after a 2-hour exposure at 40° C.; and the same thing was true for different length exposures at 30° C. Likewise, symptoms returned more quickly on plants exposed for 6 hours at 30° C. than on plants exposed for a similar period at 40° C. A definite rate for the return of symptoms on mosaic plants masked at different temperatures could not be established.

TABLE 5.—*Effect of air temperatures on masking of mosaic symptoms at Eagle, Colorado, season of 1924*

Period	Average air temperature in degrees C.		Results
	Day	Night	
May 19 to May 25	22	0	No masking
May 26 to June 1	12	−3	do
June 2 to June 8	20	0	do
June 9 to June 15	23	−3	do
June 16 to June 22	23	−3	do
June 23 to June 29	30	0	Masking
June 30 to July 6	30	5	do
July 7 to July 13	25	5	do

Influence of Soil Temperature and Soil Moisture

Experiments on soil temperatures and soil moistures were conducted in order to determine the extent to which these factors operate, if at all, in bringing about masking.

The soil temperature studies were conducted simultaneously with those on soil moisture. The Wisconsin soil temperature tanks, previously described by Jones (21), were used. Plants were grown in galvanized iron cans 5x8x12½ inches in size. These were immersed in tanks electrically heated and controlled by electric thermostats.

The moisture-holding capacity of the soil was determined by the 25 cm. tube method. The soil-moisture content was brought to the desired point by adding sufficient water to each can through a glass tube leading to an inverted porous clay pot and was kept approximately constant by weighing the cans daily and adding enough water to restore the desired weight. Increase in weight of the plants was not taken into account in the weighings.

TABLE 6.—*Duration of masking at different soil temperatures with different soil moistures (Experiment 1)*

Approximate soil temperature in degrees C.	Approximate percentage soil moisture	Results—Dec. 9 to Dec. 22, 1924	Results—Dec. 23 to Jan. 5, 1925
20	25	No masking	No masking
	40	do	do
	60	do	do
	80	do	do
25	25	No masking	No masking
	40	do	do
	60	do	do
	80	do	do
30	25	No masking	No masking
	40	do	do
	60	do	do
	80	do	do
35	25	Masking	Mosaic symptoms appeared Dec. 26
	40	do	
	60	do	
	80	do	

The soil temperature tanks were kept in a cool greenhouse in which the air temperature was held at approximately 15° C. Four tanks were used, the water in each of which was held at 20°, 25°, 30°, and 35° C. respectively. Each tank was supplied with a series of 10 cans. Five different soil moistures in duplicate were used in each tank, namely, 25, 40, 60, 80, and 100 per cent of the moisture-holding capacity of the soil.

TABLE 7.—*Duration of masking at different soil temperatures with different soil moistures (Experiment 2)*

Approximate soil temperature in degrees C.	Approximate percentage soil moisture	Results—Jan. 25 to Feb. 14, 1925	Results—Feb. 15 to Feb. 21, 1925
26	40	No masking	No masking
	60	do	do
29	40	No masking	No masking
	60	do	do
32	40	Masking	Mosaic symptoms appeared
	60	do	
35	40	Masking	Mosaic symptoms appeared
	60	do	

Triumph mosaic stock from Florida, previously indexed, was used. Two seed pieces, each composed of a single eye, were planted in each can. From each seed tuber used, four eyes were cut. All four seed pieces were planted in separate cans at the same percentage of soil moisture but held at different soil temperatures. This permitted observations on the same tuber as to the effect of variation in soil temperature.

At 20°, 25°, and 30° C., all plants showed mosaic symptoms. No symptoms appeared on plants at 35° C. until a height of 8 inches was attained, indicating that the point had been reached where low air temperature replaced high soil temperature as the dominating factor, thus inducing mosaic symptoms which earlier were inhibited. Neither high nor low soil moistures intensified or reduced the symptoms.

This experiment indicated that high soil temperature may be a factor in masking plants during the earlier stages of growth, even though the air temperature is favorable for mosaic symptoms. It also indicated that a critical temperature exists at about 30° to 35° C., above which there is masking of plants from the time of emergence until plants are about 8 inches high, and below which mosaic symptoms readily appear (table 6).

The results of other experiments were confirmatory and are given in table 7. It was found that temporary masking of leaves may be induced by soil temperatures as low as 32° C. (Plate XXXII).

No attempt was made to determine whether the amount of virus in the leaves during the period of masking was less than when characteristic symptoms were present.

Soil moisture exerted little or no influence on masking. There was practically no variation in the intensity of symptoms of plants growing in different moistures at the same temperature.

Influence of Humidity

Recorded observations concerning the effect of humidity on the symptoms of potato mosaic are very limited, judging from the literature. Mosaic symptoms have often appeared to be unusually marked in regions of high humidity, and in some cases this was attributed largely to high moisture content of the air. Other workers have considered low humidity which characterizes semi-arid and arid regions to be one of the primary contributing causes of pronounced masking. Up to the present time, no experimental evidence based on work conducted under controlled conditions has been presented.

A series of experiments was carried out in the control chambers in an attempt to determine to what extent humidity affects the symptoms. Three control chambers were operated simultaneously, the air temperature throughout all of the experiments recorded being held a few degrees below

that of the critical temperature, averaging 19° to 20° C. This temperature was favorable for mosaic symptoms. The humidity, on the other hand, was varied. Variations in humidity included relative humidities averaging 43, 81, and 95 per cent. The humidities were maintained at a fairly constant level by the control apparatus without great difficulty, never fluctuating more than 5 per cent except for a few minutes when the chambers were opened in order to water the plants or to attend to apparatus. The rate of evaporation was measured by three Livingston atmometers placed in each chamber. The method of experimenting was essentially the same as in the air temperature experiments. The results of four experiments are shown in table 8.

Neither high nor low humidities had any effect on the symptoms at the particular air temperatures used. Both healthy and mosaic plants exposed

TABLE 8.—*Influence of variation in humidity upon mosaic symptoms*

Experiment No.	Date performed	Comparative humidity in chambers	Approximate relative humidity	Average daily evaporation in c.c. from atmometers	Results
1	Nov. 12 to Dec. 2, 1924	Low	43	35.8	No masking
		Medium	81	15.9	do
		High	95	7.3	do
2	Nov. 19 to Dec. 2, 1924	Low	43	36.5	No masking
		Medium	81	15.5	do
		High	95	7.5	do
3	Mar. 2 to Mar. 15, 1925	Low	47	34.0	No masking
		Medium	81	15.2	do
		High	93	7.5	do
4	Mar. 9 to Mar. 22, 1925	Low	47	33.0	No masking
		Medium	81	13.9	do
		High	93	9.5	do

in the chambers had longer internodes than the controls grown in the cool greenhouse. Humidity in the cool greenhouse approximated that of the low humidity chamber and, inasmuch as the controls showed no internodal elongation, the conclusion seems warranted that light was largely responsible for internodal elongation in the case of plants in the chambers.

Influence of Light

Light is an important environmental factor, but one of such complexity that its relationship to normal plants is not perfectly understood, and still less is known about its relation to plant diseases. Under field conditions light is intimately associated with air temperature, and it is difficult

to attempt to separate one from the other. Certain investigators have demonstrated that the absorption of light by a green plant may raise the temperature of the plant from 4 to 10 degrees; hence any conclusions drawn regarding its effects are frequently open to serious question.

Many investigators, as previously noted, report that symptoms of potato mosaic are affected by shading the plants. The experiments on light were conducted to determine whether shading, colored lights, or constant exposure to light tends to increase or decrease the symptoms of the disease.

Effect of Shading with Cheesecloth. Four cages covered with white cheesecloth were used, varying from one to four thicknesses of cloth. Six mosaic potato plants of the People's variety were placed under each cage, while eight mosaic plants for use as controls were kept in the greenhouse without being covered. The air temperature in the greenhouse averaged about 20° C. and was therefore favorable for mosaic symptoms. Observations made over a period of four weeks indicated that mottling and wrinkling of the leaves were neither intensified nor reduced.

Effect of Colored Glass. Five glass chambers served for the study of the effect of colored light on the symptoms. A good grade of colored glass was used, the colors consisting of red, blue, yellow, and green, in addition to plain glass. Cold air was supplied to each chamber through rubber tubing by means of an electric blower which was in operation day and night. In no case did the air temperature exceed 23° C. The experiment was continued for two weeks. Mottling and wrinkling were reduced under green glass, while the other colors had no effect on the symptoms but did cause etiolation.

Effect of Constant Exposure to Light. Mosaic plants, one inch high, of the People's variety, were placed beneath five 200-watt lamps equipped with reflectors, distributed overhead in such a way as to cast essentially the same amount of light on each plant. The plants were exposed to continuous illumination, during the day from natural sources and at night by means of artificial light. Controls were kept in a remote part of the greenhouse, away from the electric lamps. The air temperature was favorable for mosaic symptoms. At the end of three weeks the internodes of plants under the electric lamps were much elongated in comparison with the internodes on the controls, while the leaves were smaller. In spite of the differences in growth between the two sets of plants, there was no macroscopic difference in symptoms between the plants given continuous light and the controls.

Other experimental results were confirmatory. The conclusion is drawn that shading with cheesecloth, colored lights, or constant illumination does not intensify mosaic symptoms, or reduce them, so far as macroscopic observations are concerned when the experiments are conducted during winter months under greenhouse conditions.

Influence of Nutrition

So far as can be ascertained, no studies have been reported on the influence of nutrition on the symptoms of potato mosaic where pure sand cultures were employed.

Johnston (20) determined the proportion of salts necessary to produce the best growth of potato plants by means of sand cultures. Potato sprouts were grown in a series of solutions consisting of 21 different salt proportions of monobasic potassium phosphate (KH_2PO_4), calcium nitrate ($Ca(NO_3)_2$), and magnesium sulfate ($MgSo_4$). He found that the average highest yielding culture contained the three salts in the following volume-molecular concentration: KH_2PO_4, 0.0065M.; $Ca(NO_3)_2$, 0.0086M.; $MgSO_4$, 0.0021M.

Nutrient solutions were prepared on the basis of Johnston's highest yielding culture. When transposed in terms of grams, the formula for his complete nutrient solution is: KH_2PO_4, 0.8850 grams; $Ca(NO_3)_2$, 1.4111 grams; $MgSO_4$, 0.2528 grams. Four solutions were prepared, consisting of a complete nutrient solution, a solution lacking in nitrogen, another lacking in phosphorus, and still another lacking in potassium.

Two sprouts, one healthy and one mosaic, from Wisconsin Triumph indexed stock, were placed in each sand culture. The sand was first flooded with nutrient solution diluted to 10 per cent strength, followed by withdrawal so as to reduce the total amount of solution to 50 per cent of the dry weight of the sand. Moisture of the sand was kept at approximately 11 per cent, which represented 50 per cent of the moisture-holding capacity as determined by the use of Hilgard trays. During the second week a new solution of 50 per cent strength was added, followed during the third week by a solution of full strength. Liquids were withdrawn from the cultures by means of suction. No attempt was made to measure evaporation or transpiration. Air temperatures in the greenhouse fluctuated from 15° to 20° C.

Four series of three crocks each were used. To the first series complete nutrient solution was added; to the second a nutrient solution lacking in nitrogen; to the third a solution lacking in phosphorus; the fourth was supplied with a solution lacking in potassium.

At the end of the fifth week the symptoms were as marked as at the beginning. There was very little difference among the several cultures as far as growth and intensity of symptoms were concerned. Other experiments were confirmatory.

These experiments, although limited in extent, were of such a convincing nature as to discourage any expectation of altering mosaic symptoms by varying the nutrition of the plants; consequently this line of effort was discontinued. The results are believed sufficient, however, to justify the

conclusion that little or no modifying influence on mosaic results from varying the three principal elements for plant growth.

Pathological Histology in Relation to Masking

Considerable attention has been given by investigators to the histology of healthy and mosaic leaves of various Solanaceae, especially tobacco, tomato, and potato.

Koning (22) and Heintzel (15) found few deviations, microscopically, in mosaic tobacco leaves from normal healthy tissue.

Woods (47), in 1902, noted that the palisade cells of the light green areas were cuboidal in shape and found there was an abrupt change from these cells to those of the dark green areas, the latter being considered healthy.

Iwanowski (17) also noted shortened and cuboidal cells in the yellow areas.

Westerdijk (46) found no striking differences between mosaic and healthy tomato leaves, while Melchers (26) observed that yellow areas were slightly thinner than the green areas in mosaic tomato leaves.

Dickson (6), in 1921, concluded that true mosaic histological symptoms included hyperplasia of palisade parenchyma in light areas. Later he (7) found a number of variations from healthy tissue in mosaic potato leaves.

The literature apparently contains no information relative to histological studies on leaves where the symptoms have been masked as a result of high temperatures. Masked leaves present an entirely different macroscopic appearance from leaves showing symptoms, particularly with regard to mottling and wrinkling. Some histological differentiation takes place, apparently, and the investigation was commenced on this assumption.

Healthy, mosaic, and masked leaves were selected during the course of both air and soil temperature experiments. Tissue was taken from masked plants at 30° C., and from mosaic and healthy leaves at air temperatures below 23° to 24° C.; in the soil temperature experiments from healthy, masked, and mosaic plants at 32° and 35° C.

All material was killed in formal-acetic-alcohol fixative and imbedded in paraffin. Sections were cut 10 microns thick and stained with Haidenhain's iron-alum-haematoxylin and the triple combination of Flemming's Safranin, Gentian-Violet, and Orange G (in clove oil).

Influence of Air Temperature. Examination of sections from healthy tissue at both 30° C. and 15° C. disclosed no abnormalities. In mosaic tissue from leaves produced at 15° C., the well-known light and dark areas were observed. Masked leaves at 30° C. have palisade cells reduced in width and length, thus eliminating all intercellular spaces, except where certain palisade cells took a position at a slightly inclined angle to the right or

left of the vertical plane of the section. The cells of the spongy parenchyma were arranged regularly in rows, and all intercellular spaces were eliminated. A leaf in a masked condition presents two marked aspects: reduction in size in both axes and compact arrangement of palisade cells, and increase in number of spongy parenchyma cells with elimination of intercellular spaces.

No attempt was made to investigate the progressive changes in histology which undoubtedly occur from the time a diseased plant is placed in a high air-temperature chamber until the time when new leaves appear devoid of all mottling and wrinkling. Under field conditions where masking has taken place and inspection for mosaic is rendered impossible, it would seem probable that, with a knowledge of the structure of healthy, mosaic, and masked leaves in mind, leaves could be examined quickly by making free-hand sections and the presence or absence of mosaic established.

Influence of Soil Temperature. Microscopically it was impossible to distinguish between healthy and masked leaf tissues of plants grown in soil temperatures of 32° and 35° C. Development of upper and lower epidermis, palisade cells, and spongy tissue with large intercellular spaces was entirely comparable to the healthy condition.

Air Temperatures in certain Potato-growing Sections. Since intermittent high air temperatures have been shown experimentally to be of considerable importance in the process of masking of mosaic symptoms under greenhouse conditions, an attempt was made to show how these results might be of practical significance in connection with conditions obtaining under the natural environment.

The data used were compiled from the records of the United States Weather Bureau and cover the potato-growing periods of the years 1915, 1916, 1923, and 1924. Six representative potato states, including two in the South and four in the North, were selected as a basis for comparison. The maximum daily temperatures were used since it was believed this would represent more correctly the conditions which concern potato mosaic from the standpoint of masking. These figures were averaged to give the air temperatures for weekly periods upon which the conclusions have been drawn. Masking, as shown by Johnson (19), occurs only above 75° F.; below that point the symptoms show readliy. The data cover the air temperatures from the approximate time of emergence of plants from the soil until harvest.

The air temperatures at Greeley, Colorado; Fort Myers, Florida; Alexandria, Louisiana; and Spooner, Wisconsin, were brought together for comparison, based on average planting dates of May 29, December 27, January 29, and June 5 respectively. A similar comparison was made between St. Augustine, Florida; Baton Rouge, Louisiana; Orono, Maine;

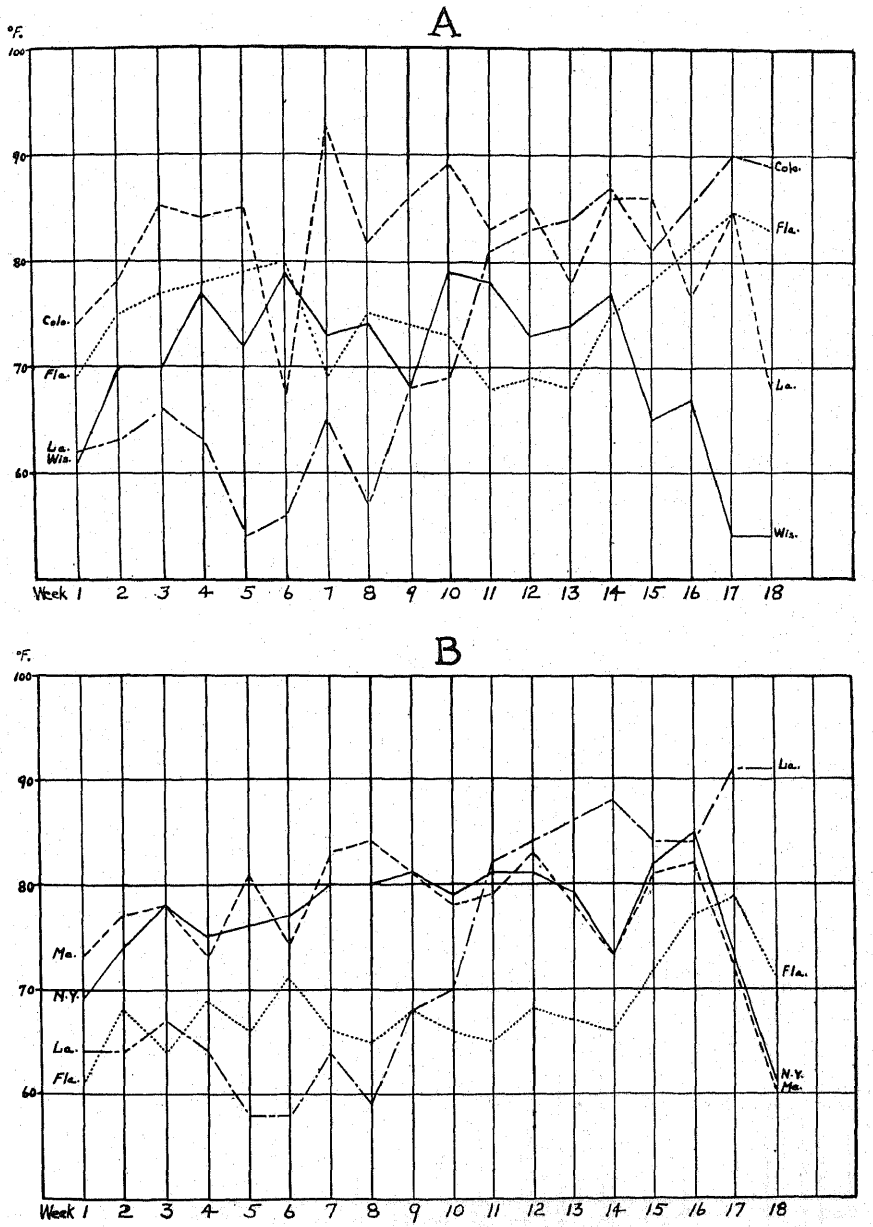

FIG. 1. Graphic comparison of average weekly maximum air temperatures prevailing in different potato-producing regions from date of planting until harvest time. 1915: A. Greeley, Colorado; Fort Myers, Florida; Alexandria, Louisiana; Spooner, Wisconsin. B. St. Augustine, Florida; Baton Rouge, Louisiana; Orono, Maine; Medford, New York.

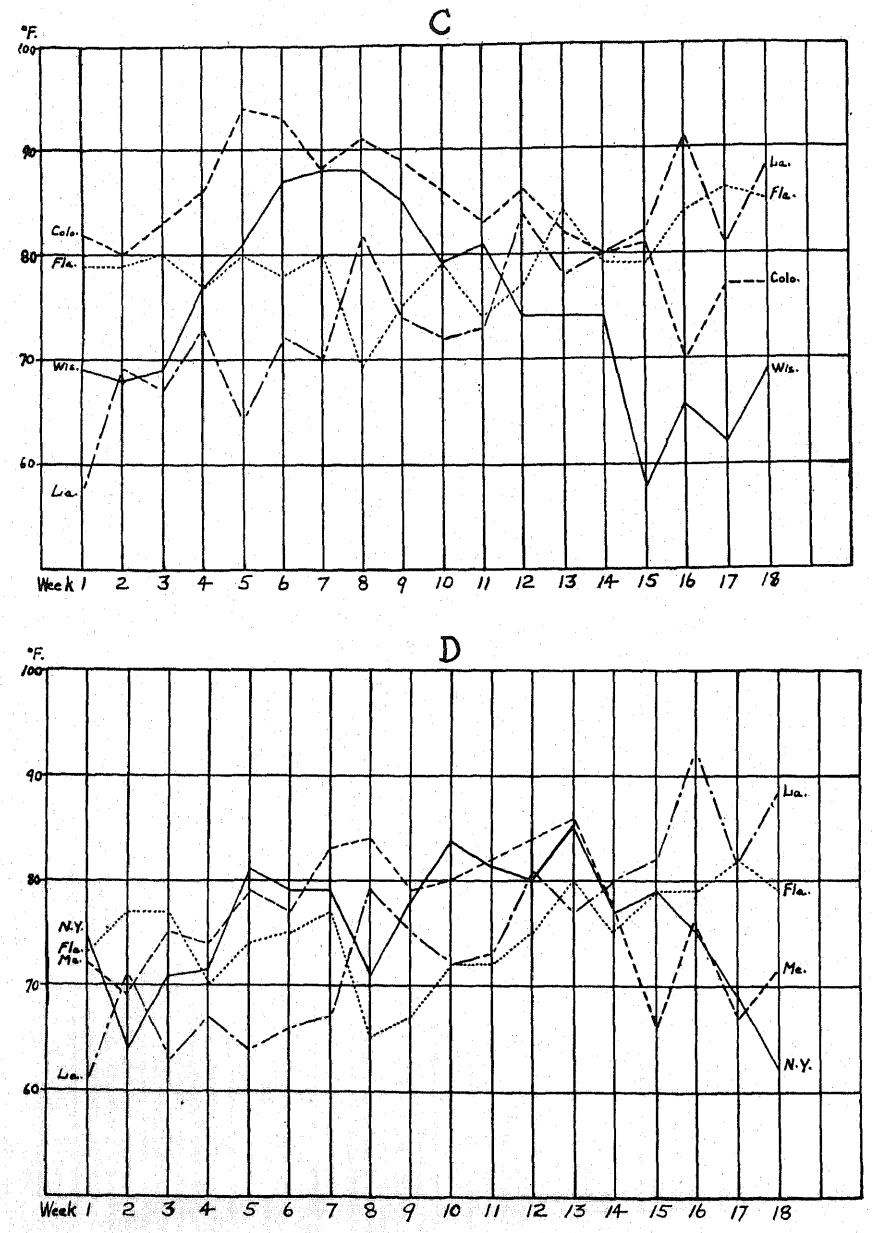

FIG. 2. Graphic comparison of average weekly maximum air temperatures prevailing in different potato-producing regions from date of planting until harvest time. 1916: C. Greeley, Colorado; Fort Myers, Florida; Alexandria, Louisiana; Spooner, Wisconsin. D. St. Augustine, Florida; Baton Rouge, Louisiana; Orono, Maine; Medford, New York.

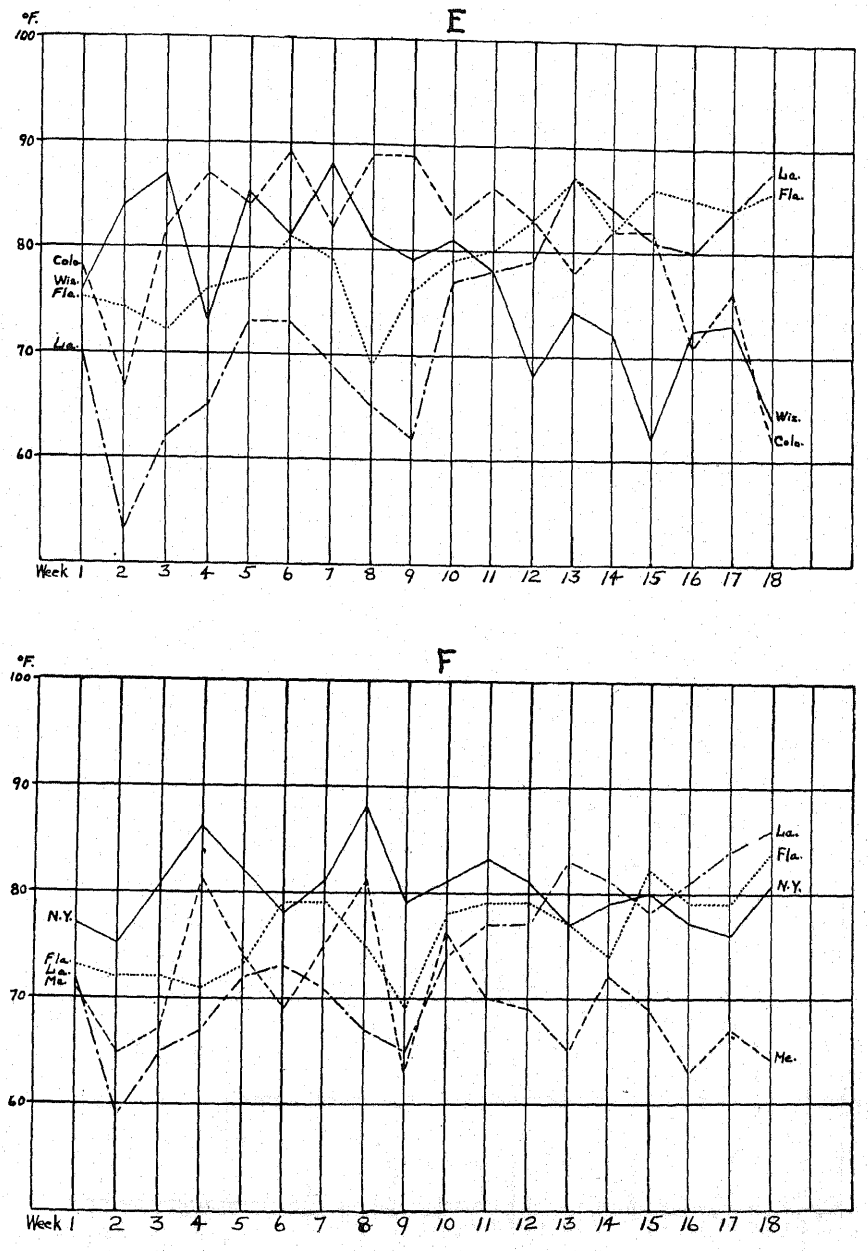

FIG. 3. Graphic comparison of average weekly maximum air temperatures prevailing in different potato-producing regions from date of planting until harvest time. 1923: E. Greeley, Colorado; Fort Myers, Florida; Alexandria, Louisiana; Spooner, Wisconsin. F. St. Augustine, Florida; Baton Rouge, Louisiana; Orono, Maine; Medford, New York.

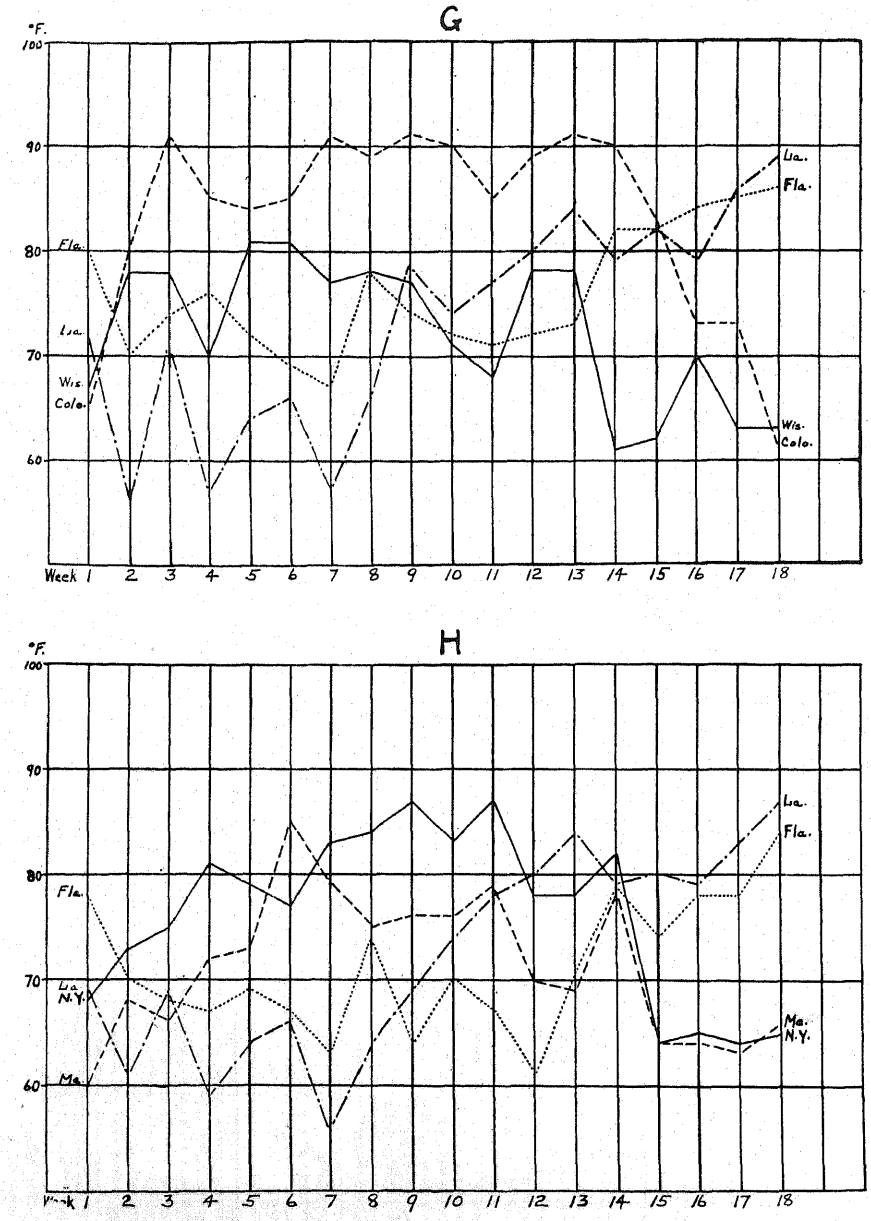

FIG. 4. Graphic comparison of average weekly maximum air temperatures prevailing in different potato-producing regions from date of planting until harvest time. 1924: G. Greeley, Colorado; Fort Myers, Florida; Alexandria, Louisiana; Spooner, Wisconsin. H. St. Augustine, Florida; Baton Rouge, Louisiana; Orono, Maine; Medford, New York.

and Medford, New York, whose average planting dates were December 27, January 29, May 29, and May 29 respectively. The air temperature records in each series are believed to be representative of conditions in the surrounding commercial potato-producing sections. The approximate dates of planting naturally do not coincide in the comparative graphs, but that is immaterial since the growing season itself was considered and not the time of year.

An attempt has been made to correlate the conclusions drawn from the climatological data with experimental results on air temperature. For example, at 30° C. (86° F.), with a 6-hour intermittent exposure, masking occurred in 6 days; while at 28° C. (81° F.) masking occurred in 7 days with a 9-hour intermittent exposure. Masking undoubtedly proceeds at a

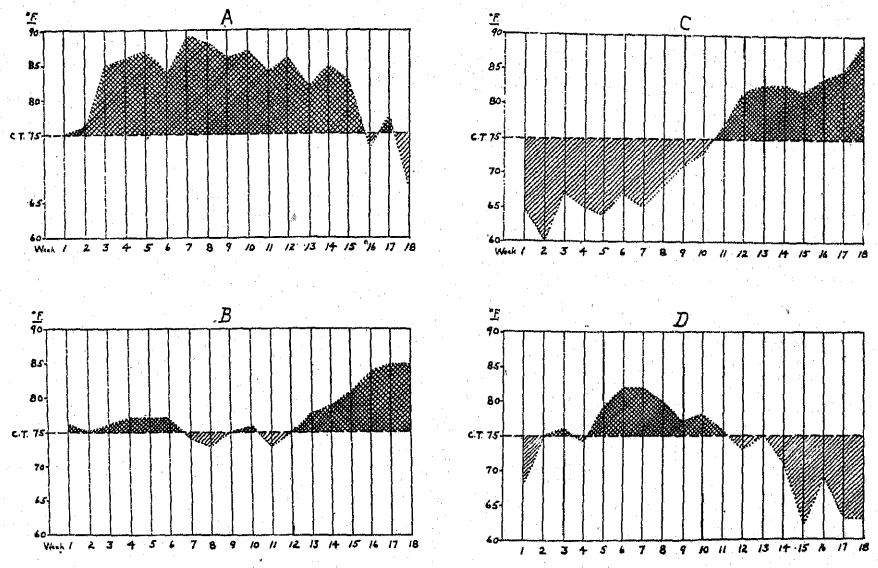

FIG. 5. Graphic representation, based on a 4-year average of maximum daily air temperatures, showing periods favorable for masking of symptoms in different potato-producing regions. A. Greeley, Colorado. B. Fort Myers, Florida. C. Alexandria, Louisiana. D. Spooner, Wisconsin.

more rapid rate in the field because the symptoms are less marked outdoors than on diseased plants produced under a more uniform air temperature in a greenhouse. Employing the six-hour exposure as a basis of comparison, figures 1, 2, 3, and 4 illustrate differences in temperatures in the several states, which may or may not mask symptoms, for each of the four years. An interpretation of the four-year averages is presented in figures 5 and 6, which illustrate the periods during the growing season when high temperatures favorable for masking occur. In Colorado the period of

masking is most likely to begin about the third week and continue throughout the entire growing season. The latter half of the growing season in Florida and Louisiana, from the tenth week on, favors masking. In Maine, New York, and Wisconsin, the second one-third of the season, from the fourth to the thirteenth week, is favorable for masking of the symptoms.

DISCUSSION OF RESULTS

It is believed that the experimental evidence presented establishes be-

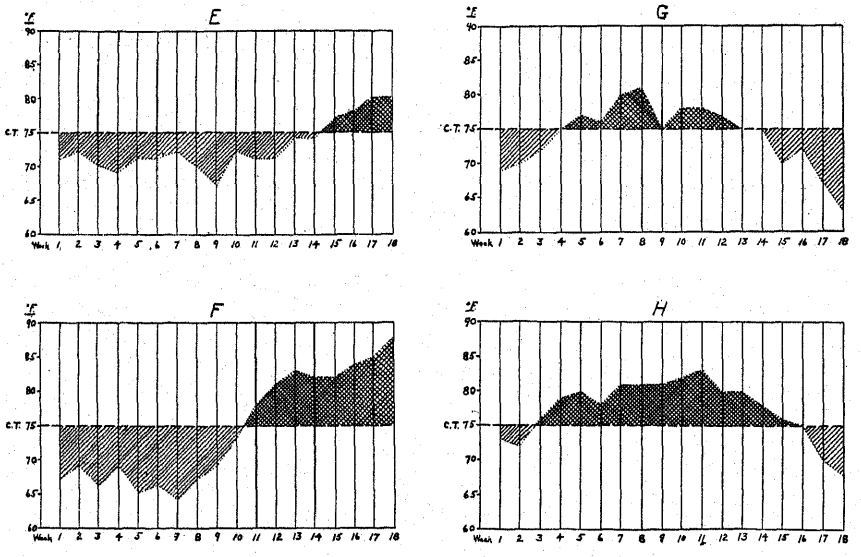

FIG. 6. Graphic representation, based on a 4-year average of maximum daily air temperatures, showing periods favorable for masking of symptoms in different potato-producing regions. E. St. Augustine, Florida. F. Baton Rouge, Louisiana. G. Orono, Maine. H. Medford, New York.

yond reasonable doubt that air temperature is the criterion which influences the presence or absence of the symptoms of potato mosaic and that such factors as soil temperature, soil moisture, humidity, light, and nutrition are of minor, if any, importance.

Relatively short intermittent exposures at air temperatures above the critical temperature (23° to 24° C.) are sufficient to induce masking of the symptoms, the rate of masking depending largely upon the length of exposure, upon the air temperature to which mosaic plants are exposed at or above the critical temperature, and upon the age of the leaves. The experimental results, therefore, indicate the importance and significance of the effect of relatively short periods of temperatures above 24° C. on mosaic,

especially when comparison is made between the data obtained under controlled conditions and that secured from official weather records.

The application in research and practice of the experimental results on intermittent exposures at high air temperatures is obvious. Experimental studies with mosaic should not be carried out without giving attention to the air temperatures at which they are performed. This is especially true in cross inoculation experiments and in studies on the influence of other environmental conditions or circumstances on the occurrence of mosaic.

The tuber-index method of eliminating mosaic from seedstock as described by Blodgett (1) will no doubt be used to an increasing extent. It is fundamental to this method that attention be given to the air temperature at which the potatoes are grown.

Of greater importance at present is the relationship which intermittent exposures at high air temperatures bear in general to seed potato inspection and certification work. At best, tuber indexing cannot be carried out on a large scale owing to the limited amount of available greenhouse space and to the relatively short period of time in which this type of work can be done, limited as it largely is to the winter months. Consequently the major part of the seed-improvement work must be done under a system of roguing in seedplots or in the commercial field. The success or failure of this method is directly dependent upon the daily variation in temperature. The possibility of great variation in several northern and western states with respect to periods favorable for the appearance of symptoms of potato mosaic has been suggested in a preceding chapter. In the West, the difficulties are apparently much greater than in the middle-western or eastern states, owing to periods of prolonged high air temperatures.

The effect of short exposures at high air temperatures on potato plants growing under field conditions also presents another aspect of general interest and one of much economic importance. The northern and western states which devote considerable acreage to the Triumph variety depend largely upon southern states as a market for seedstock. It has previously been shown that low air temperatures may predominate throughout the first half or more of the growing period in southern potato regions, with the result that the northern and western certified seed or other seedstock is put to a severe test in the average season. The southern grower is therefore naturally desirous of securing as healthy seed potatoes as possible from the seed-potato growing districts. In some seasons northern stock has apparently been entirely satisfactory, but, at other times, as a whole it has not. This may be due, in part at least, to seasonal differences in air temperatures which have been unfavorable for eliminating diseased stock in the North or which have been especially favorable for bringing out the symptoms in the South. In Louisiana and other southern areas, in the

spring of 1925, it has been estimated by experiment station officials that an average of 20 per cent of mosaic appeared in fields planted from certified stock, while the mosaic count in fields planted from common stock varied from 20 to 90 per cent. In consequence the yield of potatoes in the South is reduced considerably, and this in turn reflects on the market for the northern seed-potato producer. Reduction in the amount of mosaic in northern stock in the future will apparently depend largely upon the amount of seed which can be properly indexed under greenhouse conditions and the increase of this stock at suitable isolated places rather than upon roguing in the field, although it is recognized the latter method may always be a necessary adjunct.

SUMMARY

1. Studies have been made to determine the influence of air temperature, soil temperature and soil moisture, humidity, light, and nutrition on the symptoms of potato mosaic.

2. Air temperature was found to be the main and only factor affecting the amount and intensity of symptoms on potato foliage under greenhouse conditions.

3. Experiments conducted in constant air-control chambers indicated that relatively short exposures to air temperatures above that of the critical air temperature (23° to 24° C.) were sufficient to mask the symptoms of mosaic. The rate of masking was found to be dependent largely upon the duration of exposure, upon the air temperature to which exposed, and upon the age of the leaves.

4. Differences in air temperature accounted for marked variation in leaf area produced on mosaic plants exposed for the same length of time.

5. Masked mosaic plants subjected to low air temperatures always redeveloped characteristic symptoms of mosaic.

6. Symptoms of mosaic on the Triumph variety appeared in the field at a high altitude when air temperatures were favorable, but mottling was not as marked as under low altitude conditions.

7. High soil temperatures may be of importance in masking mosaic plants during the earlier stages of growth, regardless of the amount of moisture present in the soil, or air temperature favorable for the appearance of symptoms.

8. The effect of soil moisture, humidity, light, and variation in three essential elements for plant growth failed to decrease or intensify the symptoms of mosaic potato plants.

9. Histological studies indicated that masked leaves show marked deviation in structure from healthy leaves.

10. The time and duration of the period of masking varies in different potato-producing regions.

11. Intermittent air temperatures are of fundamental importance in connection with the tuber-index method for testing seed potatoes, in seed potato inspection and certification, and in experimental studies on mosaic.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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EXPLANATION OF PLATES

PLATE XXVIII

Representative leaves (B, C, D) from potato plants exposed for 15 hours at night to temperatures indicated, but remaining for 9 hours with controls (A and E) at about 15° C.

A. Mosaic control.
B. Mosaic leaf at 15° C. showing typical symptoms.
C. Mosaic leaf at 24° C. completely masked after 8 days.
D. Mosaic leaf at 28° C. completely masked after 8 days.
E. Healthy control.

Representative leaves (F, G, H) from potato plants exposed for 9 hours daily to temperatures indicated, but remaining for 15 hours with controls (A and E) at about 15° C.

F. Mosaic leaf at 15° C. showing typical symptoms.
G. Mosaic leaf at 24° C. partially masked after 7 days.
H. Mosaic leaf at 28° C. completely masked after 7 days.

PLATE XXIX

Representative leaves from potato plants exposed for 4 hours daily to temperatures indicated but remaining for 20 hours with control plants D and E at about 15° C.

A. Masking almost completed in mosaic leaf at 28° C. after 15 days.
B. Mosaic leaf at 24° C. with severe symptoms partially lacking.
C. Mosaic leaf at 15° C. showing typical symptoms.
D. Healthy control.
E. Mosaic control.

PLATE XXX

Representative mosaic potato plants exposed for 2 hours daily to temperatures indicated, but remaining for 22 hours with control plant D at about 15° C.

A. Mosaic plant at 15° C. showing typical symptoms.
B. Mosaic plant at 24° C. showing typical symptoms.
C. Mosaic plant at 28° C. almost completely masked after 25 days.
D. Mosaic control.

PLATE XXXI

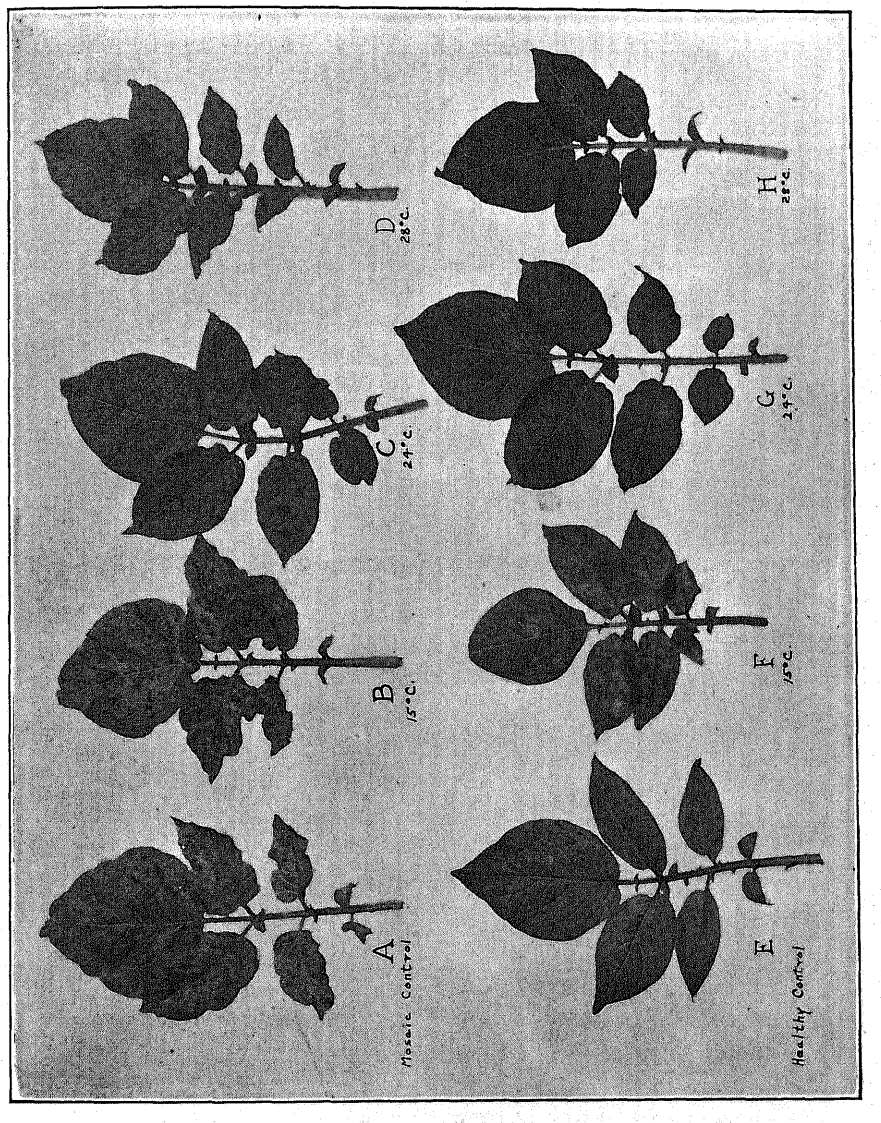
Representative mosaic potato plants exposed for 6 hours daily to temperatures indicated, but remaining for 18 hours with control plants A and B at about 15° C.

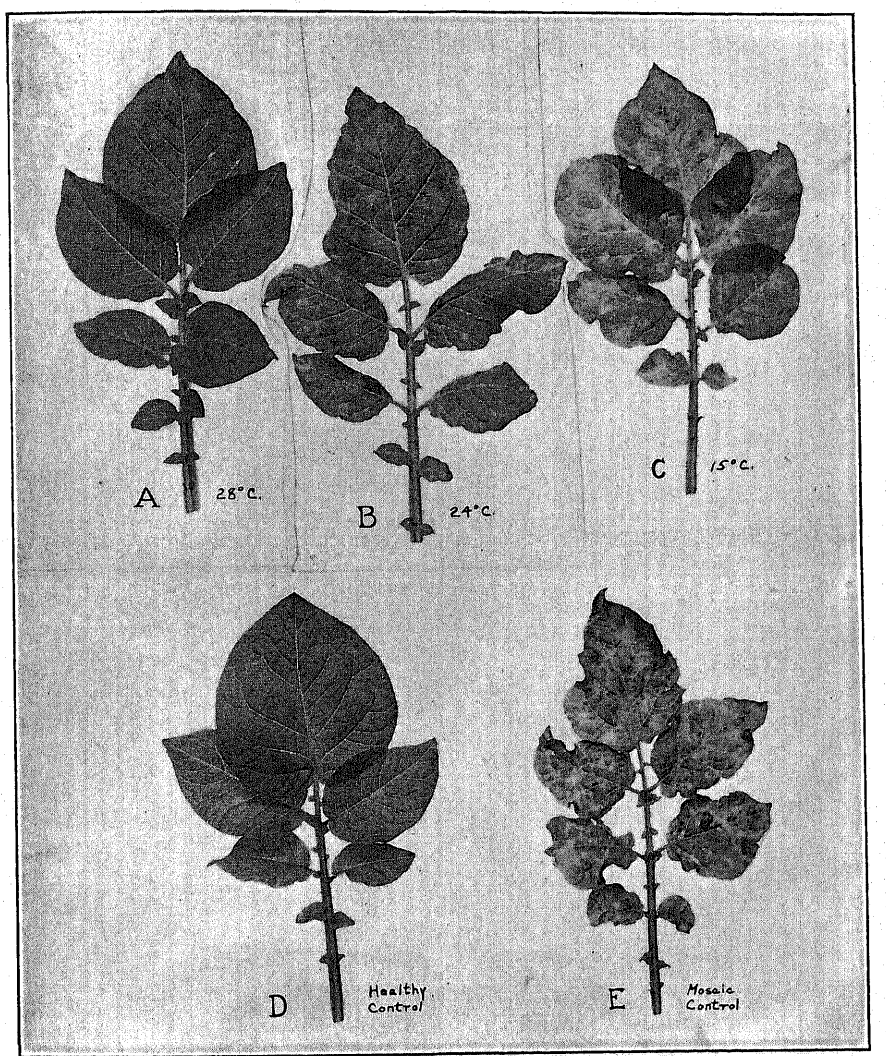
A. Healthy control.
B. Mosaic control.
C. Mosaic plant at 40° C. with symptoms masked after 6 days; decided stunting.
D. Mosaic plant at 30° C. with symptoms masked after 6 days.
E. Mosaic plant at 20° C. showing typical symptoms.

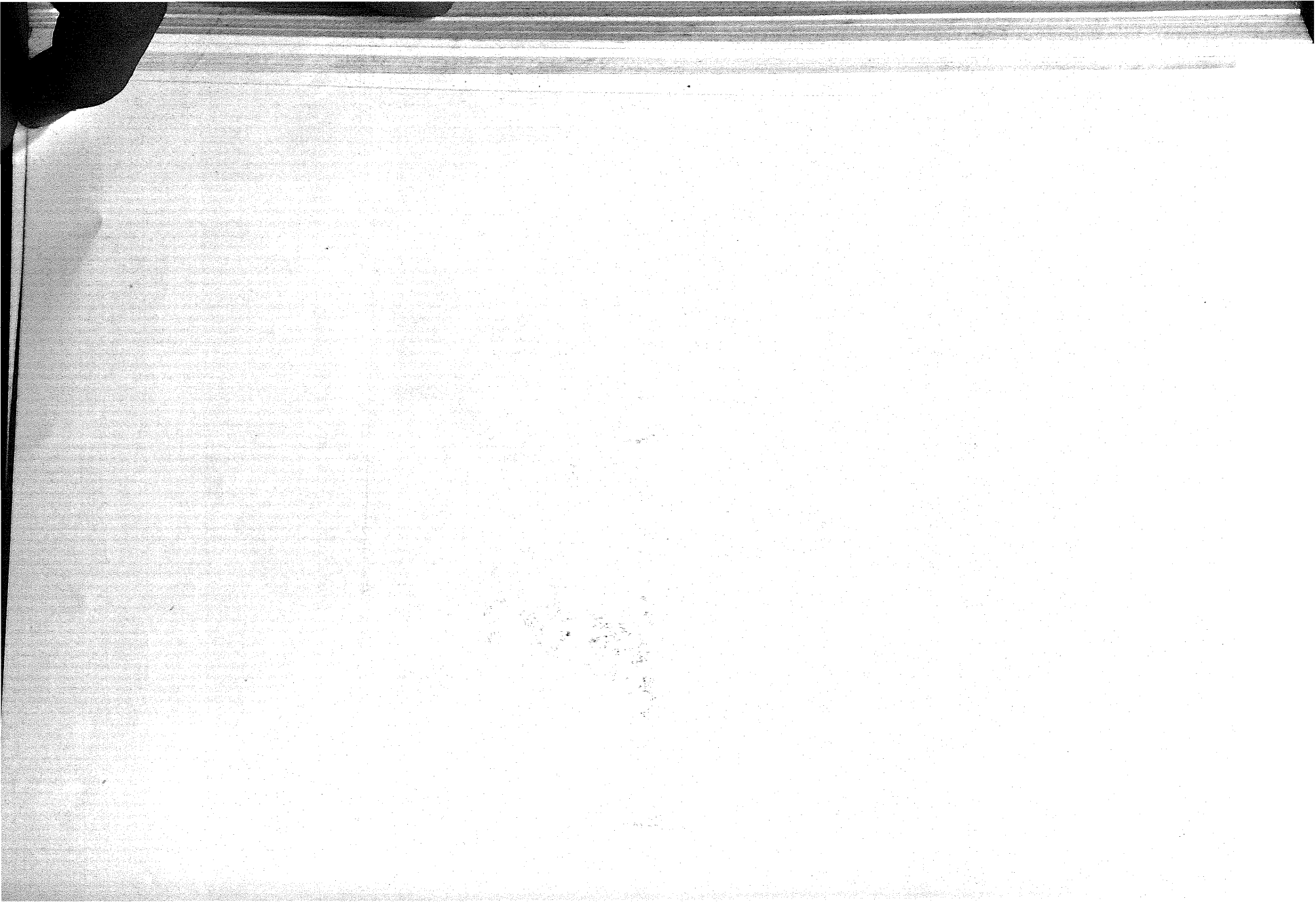
PLATE XXXII

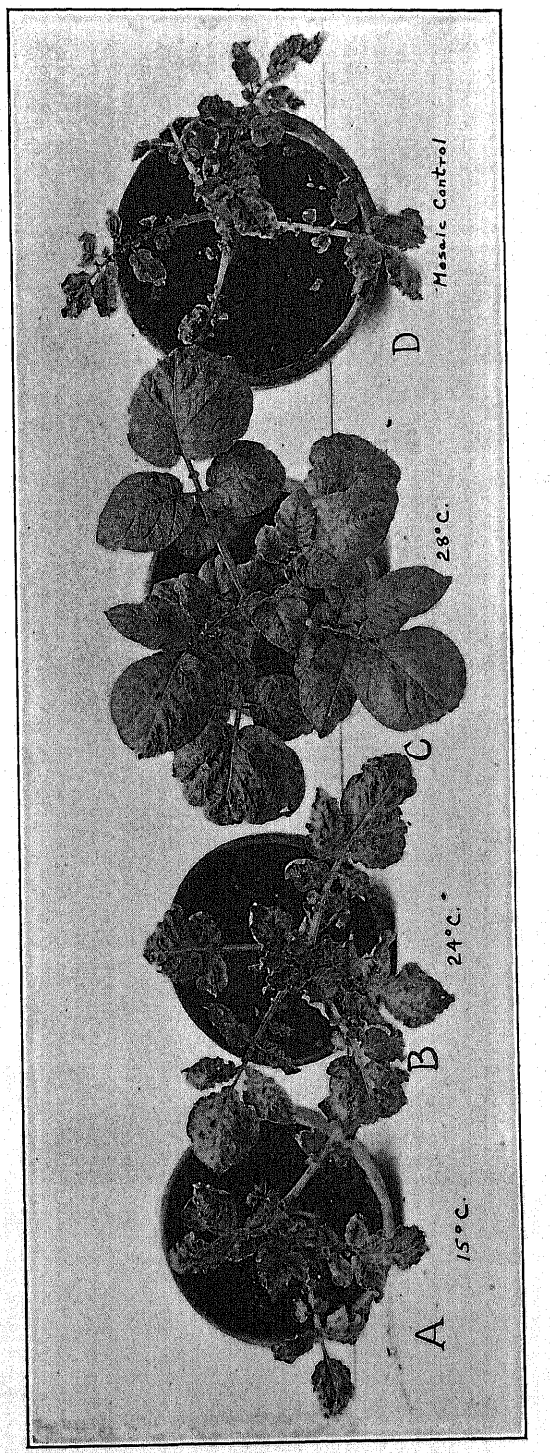
Representative healthy and mosaic potato leaves from plants grown in soil temperatures indicated, but at air temperatures favorable for mosaic.

At 26° C.
A. Healthy leaf.
B. Mosaic leaf showing typical symptoms.
At 29° C.
C. Healthy leaf.
D. Mosaic leaf showing typical symptoms.
At 32° C.
E. Healthy leaf.
F. Mosaic leaf with symptoms completely masked after 18 days.
At 35° C.
G. Healthy leaf.
H. Mosaic leaf with symptoms completely masked after 18 days.

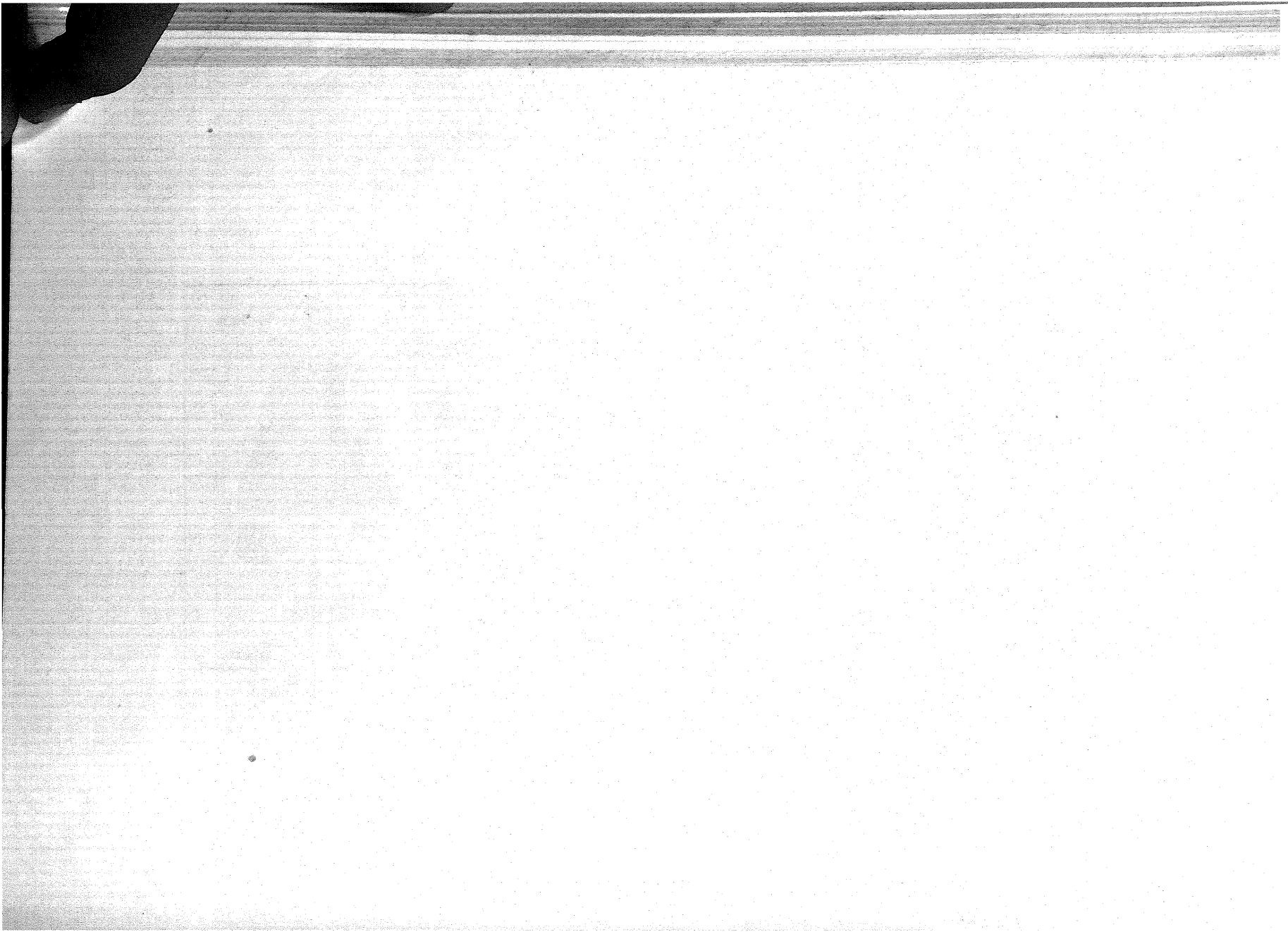

A
Mosaic Control
B
15°C.
C
24°C.
D
28°C.
E
Healthy Control
F
15°C.
G
24°C.
H
28°C.

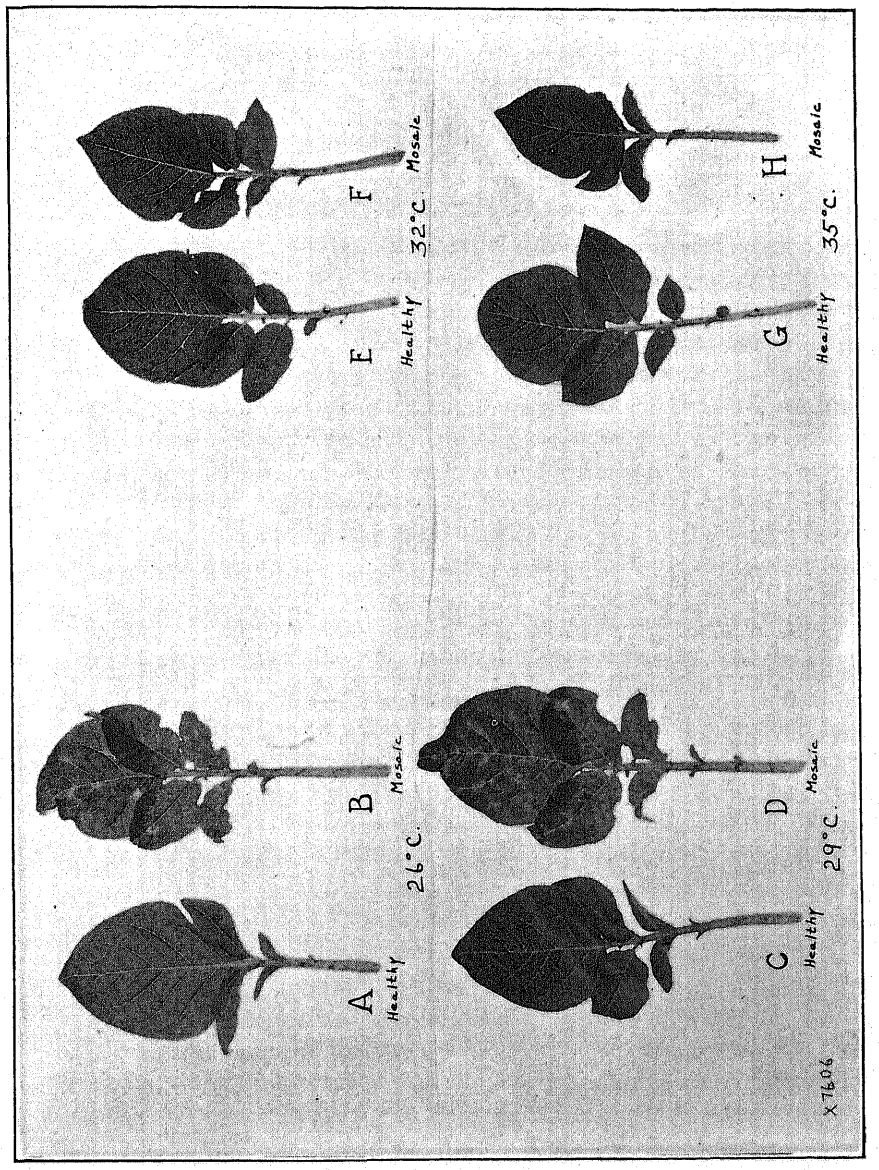

A
28°C.
B
24°C.
C
15°C.
D
Healthy
Control
E
Mosaic
Control




A
15°C.
B
24°C.
C
28°C.
D
Mosaic Control

X7056
A
Healthy Control
B
Mosaic Control
C
40°C.
D
30°C.
E
20°C.




A
Healthy
B
Mosaic
26°C.
E
Healthy
F
Mosaic
32°C.
C
Healthy
D
Mosaic
29°C.
G
Healthy
H
Mosaic
35°C.
X 7606

VERTICILLIUM-WILT OF MAPLE AND ELM-SEEDLINGS IN HOLLAND

JIKKE H. H. VAN DER MEER

ACER

Verticilliosis is described as a serious disease of several species of *Acer* in the United States of America and in Canada [Rankin (6), Zimm (7), Colley (4), Anonymous (1)]. According to Zimm (7), the isolated fungus appeared to be entirely different from *Verticillium albo-atrum* attacking potato. As he, and also Rankin (6), mentioned sclerotium-like bodies, it is highly probable that the fungus in question was *Verticillium dahliae* Kleb.

A case of Verticillium-wilt of maple was observed in Holland in 1919 by the Phytopathological Service, and the disease was attributed to *Verticillium albo-atrum* (2). In March, 1925, the Phytopathological Service, receiving some diseased young maples from another locality, sent one of the specimens to me. All isolations in this case gave *Verticillium dahliae* Kleb.

In September, 1925, the opportunity of studying maple-wilt more exactly offered itself. In the garden of a laboratory situated on the "Wageningsche Berg," where previously many cases of Verticilliosis, occurring in a great variety of hosts, were observed and described (5), two specimens of *Acer platanoides* L. var. *schwedleri,* about nine years old, suddenly showed the characteristic symptoms of wilting. One of the trees was photographed September 22 (Fig. 1). Five of the branches were dead, two were defoliated, five bore only a few leaves, and two had more normal foliage. In the part of the crown which was still living there were several dead lateral branches. The tree had produced very little new growth; the one-year-old shoots were generally 15–24 cm. and those of one branch were 40 cm. long. Four suckers had developed at the trunk, two of which bore wilting leaves. The north side of the trunk was flattened from the base to the top, the bark having died and ruptured longitudinally, especially where it touched the living tissue. Also at the other side of the tree, and at the bases of some branches, dead sunken areas were to be seen. The conidial stage of *Nectria cinnabarina* occurred here and there on the injuries. The canker already had been noticed the preceding year. The dead areas were then cut out and the wounds dressed with "carbolineum." No wonder that at the outset the gardener attributed the disease to *Nectria.* Being not quite sure of the diagnosis, he drew my attention to the trouble.

The tree was dug September 23 and studied in detail. The cross-sections of trunk, branches and roots (Pl. XXXIII, A) showed a characteristic discoloring of the wood; a central, irregular brown-green spot with a fringed dark-green edge, whereas stipples of the same color were found in the spring wood of the youngest annual rings. The internal symptoms resembled closely those of wilting cherry trees, except in the color of the diseased wood, which in the latter case was brown (5). The annual ring of 1925

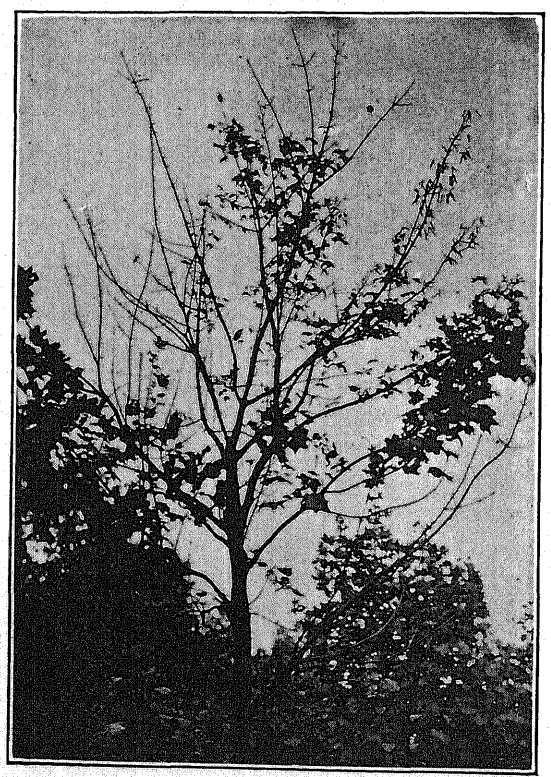


FIG. 1. *Acer platanoides* L. var. *schwedleri* at Wageningen naturally infected with *Verticillium dahliae* Kleb. Photographed Sept. 22, 1925.

was much less well developed than the others and it failed completely at the above mentioned cankered spots (Plate XXXIII, A). The discoloration of the 1924 and 1925 annual rings could be followed in a longitudinal direction from the roots up to some two-year-old shoots. In one case a slight discoloration was still observed even in the current season's growth.

The trunk, the branches and the root, of which cross-sections are photographed, were subjected to cultural and microscopical examination. *Ver-*

ticillium dahliae Kleb. was isolated from the woody tissue of the three different parts of the tree. It grew out chiefly from the vessels of the 1925 annual ring. It appeared that a two-year-old shoot already contained the fungus, the new growth not yet being attacked.

Sections were made of the diseased wood, and a microscopic examination was made. The vascular walls were yellowed, and a gum-like substance was frequently noticed within the vessels. Radial sections were prepared from the remnants of those branch and root pieces from which *Verticillium dahliae* had been isolated. These sections were stained with Bleu cotton and the mycelium could be observed readily in the vessels. Microsclerotia mentioned by Rankin (6) were not found in the tracheae.

ULMUS

According to Versl. en Med. Phytopath. Dienst Wageningen (2) *Verticillium* was isolated from the branches of *Ulmus wheatlei,* the wood of which had brown stipples. The disease was also observed in *Ulmus campestris.*

Bewley (3) inoculated six young elm seedlings with *Verticillium* isolated from tomato. After four months they were shorter than the controls, but he did not observe any other symptoms.

In October, 1925, Verticilliosis developed very clearly among one-year-old seedlings of *Ulmus campestris* L. on an experimental plot situated on the "Wageningsche Berg." Miss Spierenburg, phytopathologist at Wageningen, isolated *Verticillium dahliae* and drew my attention to the disease. Previously potatoes had been grown on the field, which in this way had become infested with *Verticillium.*

Several specimens showed the typical yellow blotches on the leaves and the plants were stunted and partially defoliated. Five seedlings were dug and used for study.

On cross-sectioning roots, stems and branches, the characteristic browning of the wood was seen (Pl. XXXIII, B). The discoloration could be followed to the finest tips of some branches. *Verticillium dahliae* Kleb. was isolated from the roots, stems and branches, even from the top of a shoot.

Microscopical examination showed that the vascular walls were yellowed, and the vessels contained a gum-like substance and tyloses. By staining longitudinal sections of stem and root with Bleu cotton, mycelium was detected within the vessels.

SUMMARY

1. Verticilliosis, caused by *Verticillium dahliae,* was found on young trees of *Acer platanoides* var. *schwedleri,* and seedlings of *Ulmus campestris.*

2. The organism was isolated from roots, trunks, and branches of the infected plants.

3. The disease on maple is characterized by defoliation and death of branches, the production of suckers and cankers, and a brown-green discoloration of the wood. The elm seedlings are stunted and partially defoliated and the wood becomes brown.

4. Gum-like substances and mycelium were found in the vessels of diseased wood. There was a yellowish discoloration of the walls of the vascular elements.

INSTITUTE FOR PHYTOPATHOLOGY,
LABORATORY FOR MYCOLOGY AND POTATO RESEARCH,
WAGENINGEN, HOLLAND.

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EXPLANATION OF PLATE XXXIII

A. Cross-sections of stem, branches and root of the maple represented in Fig. 1.

1a. The base of the stem.
1b. ± 95 cm. higher than 1a.
1c. ± 1.34 m. higher than 1b.
2a. The base of a branch.
2b. ± 55 cm. higher. } dark-green stipples in 1925 annual ring
3a. The base of a branch.
3b. ± 25 cm. higher. } do
4a. The base of a root.
4b. ± 35 cm. from the base.
Photographed Sept. 23, 1925.

B. Cross-sections of the stem and three branches of a one-year-old seedling of *Ulmus campestris* L. naturally infected with *Verticillium dahliae* Kleb. Photographed Oct. 14, 1925.

A

1a

1b

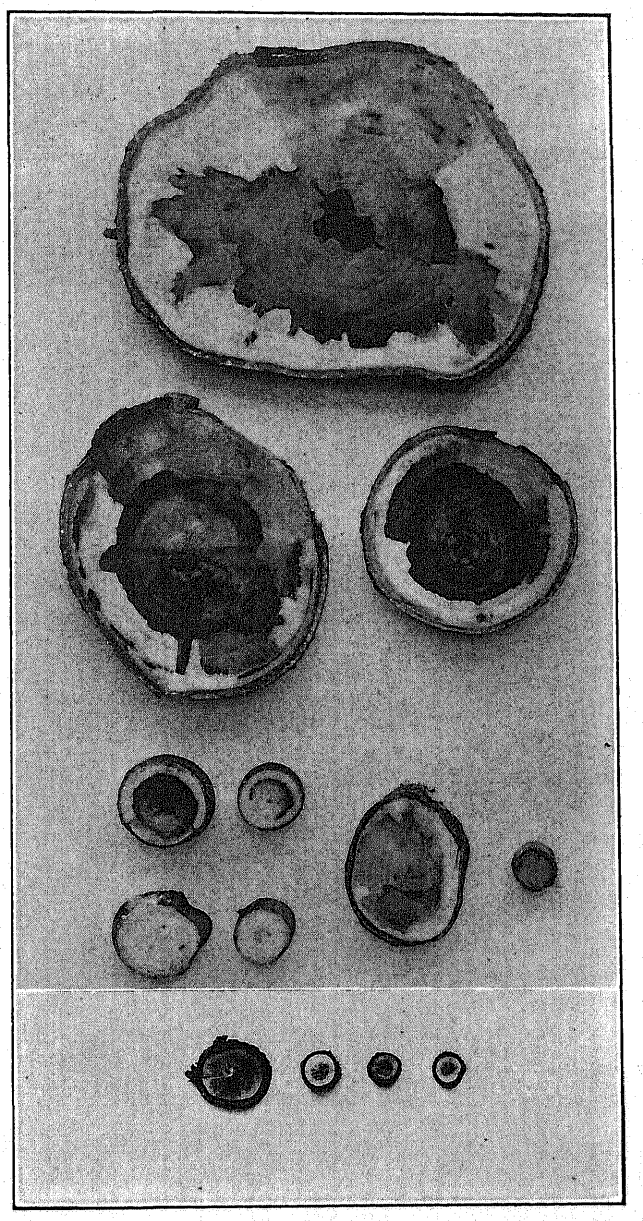
1c

2a b

3a b

4a b

B



THE CAUSE OF THE PERSISTENT DEVELOPMENT OF BASAL SHOOTS FROM BLIGHTED CHESTNUT TREES[1]

ARTHUR HARMOUNT GRAVES

In many places in the blight-devastated area of the American chestnut, the notion seems to have been gaining prevalence that the chestnut is "coming back"; since, frequently, where the main trunks have been killed off by the blight, vigorous shoots are seen to arise from the base of the tree. When these are killed by *Endothia parasitica,* as usually happens in a few years, fresh shoots appear, and successive generations of these basal sprouts may continue to develop for a long period. Cases are known to the writer where such crops of basal shoots have been periodically growing and dying for more than 15 years.[2] The vigor of these shoots, as well as the fact that they appear so persistently after the supposed death of the tree, has doubtless given rise to the popular belief in a general resurrection of the chestnut.

As an explanation of this apparent rehabilitation, it would seem indicated *a priori* that the tissues of the root collar (*i.e.,* the part of the tree where root and trunk unite), and of the roots of the chestnut, are more resistant to the growth of the fungus than the parts above ground. For when the fungus reaches these basal parts after killing the trunk above, it apparently grows more slowly, giving time for adventitious buds to develop, and, nourished by the still healthy roots of the old tree, to form vigorous young shoots. In order to test out this hypothesis, it was determined to get some definite data on the comparative resistance of root and stem tissues. For this purpose inoculations of healthy roots and basal shoots of blighted trees were made in August, 1924, at Hamden and at West Hartford, Connecticut. The inoculum used was a pure culture of *Endothia parasitica* isolated from a dying tree at West Hartford, Connecticut. All inoculations, whether on root or shoot, were covered immediately with white adhesive tape. Immediately after the roots were inoculated they were covered, in some cases with dead leaves, on which the original earth was piled but not packed down; in other cases the earth was replaced directly on the roots. On inspection one year later, no difference in the rate of

[1] Investigations carried on by the Brooklyn Botanic Garden in cooperation with the Office of Investigations in Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture. Brooklyn Botanic Garden Contributions No. 47.

[2] As a matter of fact, the condition is not quite so simple as this. Fresh shoots may appear each year, and at the same time older ones may die out, so that we do not usually find a well-defined series of crops.

growth of the fungus was noted as a result of these two methods of treatment.

The results of the inoculations after one year's growth of the fungus are given in table 1. A general inspection of table 1 shows at once that the fungus grew much more slowly in the roots than in the shoots. In fact its growth in many of the inoculated roots is surprisingly small. In every case, however, inspection showed the mycelium to be present in the bark near the inoculation slit, and in one case (No. 20) a small root had been girdled, showing the fruiting pustules of the fungus. Where the lateral growth of the fungus was less than 0.4 mm, it was classed as "slight."

An interesting feature of the inoculated roots was the sunken character of the lesion. In other work by the writer on resistant chestnut (6), hypertrophy of the host tissue in the region of the lesion, at least in the parts above ground, has been noted as a common occurrence. The writer is unable to offer any explanation for the opposite effect in the root tissue, except that the rate of growth of the root tissue may be comparatively slow. A fact that may have some relation to this behavior is that in many cases the mycelium of the fungus had invaded the outer root bark only, the inner bark tissues being white and sound.

The shoots were all girdled without exception, and, of course, where they were small, the circumference of the stem does not represent the limit of the potential growth of the fungus. Nos. 27–29, however, very closely approximated the average annual lateral growth of the fungus as given by Anderson and Rankin (1) (15.97 cm.) for Charter Oak, Penn.; by Rogers and Gravatt (7) (16.13 cm.) for Bluemont, Va.; and by Stevens (8) (16 cm.) for Hartford, Conn.

The following roots and shoots of approximately equal circumference may be compared; nos. 30 and 31 of the shoots with nos. 1, 29, and 36 of the roots; nos. 10, 17, 18, 19, and 24 of the shoots with no. 34 of roots; and nos. 25 and 26 of the shoots with nos. 2, 3, 7, 8, 10, and 11 of the roots.

The cause of the greater resistance of the root tissue may be due, at least in part, to the presence of some substance or substances which are either lacking in the shoots or present in smaller quantities than in other parts of the tree. It is possible that these substances are tannin compounds or substances associated with tannin, which it has been demonstrated experimentally (2, 4, 5) have an inhibitory effect on the growth of the fungus, when above a certain concentration. That the roots of the chestnut contain a much higher tannin content than the trunk has been shown by recent studies of the Leather and Paper Laboratory of the Bureau of Chemistry,[3]

[3] Unpublished paper entitled "The distribution of tannin in the American chestnut tree. Utilization of chestnut stumps." Leather and Paper Laboratory, Bur. of Chemistry, U. S. Dept. Agr.

TABLE 1.—*Comparative growth of Endothia parasitica (Murr.) And. in roots and shoots of Castanea dentata for one year ending August 8–10, 1925*

No. of tree[a]	Shoot inoculations			Root inoculations		
	Inoculation number	Circumference of shoot in cm. at region of inoculation	Lateral growth of fungus	Inoculation number	Circumference of root in cm. at region of inoculation	Lateral growth of fungus in cm.
I	1	3.7	girdled	1	13.8	slight
	2	3.7	do	2	11.6	do
	3	4.7	do	3	10.1	3.0
	4	4.4	do	4	8.8	slight
	5	3.7	do	5	8.8	4.0
	6	4.4	do	6	Main trunk root	4.0
	7	3.7	do	7	11.6	1.0
	8	3.5	do	8	11.6	slight
II	9	5.3	do	9	8.8	2.2
	10	2.5	do			
III				10	11.0	1.5
				11	11.0	2.0
	11	3.7	do	12	8.8	1.5
	12	3.7	do	13	6.9	1.6
				14	7.9	1.8
IV	13	4.7	do	15	7.9	2.5
	14	4.7	do	16	8.8	1.2
	15	3.7	do	17	7.9	0.8
	16	3.7	do	18	28.0	1.7
	17	2.5	do	19	31.7	2.3
V				20	1.6	girdled
				21	1.6	slight
				22	1.6	0.4
	18	2.8	do	23	1.6	0.4
	19	2.5	do	24	7.9	0.4
				25	7.9	0.8
				26	1.6	slight
VI	20	4.7	do			
	21	2.7	do	27	23.9	1.4
	22	4.0	do	28	23.9	1.2
	23	4.0	do			

TABLE 1.—*Continued.*

No. of tree[a]	Shoot inoculations			Root inoculations		
	Inoculation number	Circumference of shoot in cm. at region of inoculation	Lateral growth of fungus	Inoculation number	Circumference of root in cm. at region of inoculation	Lateral growth of fungus in cm.
VII	24	2.5	girdled	29	13.8	4.0
				30	13.8	slight
				31	7.9	do
				32	8.8	do
VIII	25	10.4	do	33	8.8	do
	26	10.0	do	34	2.5	do
				35	21.0	do
				36	13.8	do
	27	17.0	do			
	28	16.0	do	37	Large main root	0.4
IX	29	15.4	do			
	30	14.5	do	38	22.0	2.8
	31	14.5	do	39	22.0	1.7

[a] Nos. I–VII, Hamden, Conn.; nos. VIII–IX, West Hartford, Conn.

and also by analyses made in the same laboratory in cooperation with this office. In particular, the bark of chestnut roots is found to be high in tannin, containing, in the samples analyzed, more than twice the amount found in the bark of the trunk.

After a careful study of all stages of these basal shoots, their life history appears to be about as follows: during and after the death of the trunk, adventitious buds, which are normally abundantly present in the root collar of the chestnut, begin to develop into shoots, utilizing the main root of the tree, which still may be healthy in certain parts, for their support from the soil. But very soon small new roots develop at the base of these shoots. Where the adventitious buds were a few inches above the ground, I have found aerial roots growing down toward the soil, but in most cases the buds are located far enough down so that the roots start in the soil. In time, as the root of the old tree dies, these new roots become larger and eventually become the sole support of the shoot (Fig. 1). Sooner or later, however, the blight appears—usually at the ground level—ending the life of this particular shoot.

In the meantime, the shoots, if not diseased above, may have lived 4, 5 or 6, or even more years; and I have seen them 20 feet high, in some cases

FIG. 1. Basal shoots with their new roots from the region of the root collar of an old chestnut stump. The part of the stump above the shoots has been dead at least 10 years. Lying across the top of the stump is a part of a dead shoot belonging to a former generation of sprouts. The shoot to the extreme right is also now dead.

bearing catkins and maturing nuts. In the interim other shoots have been developing from adventitious buds on the old stump. After the death from Endothia infection of a large shoot with correspondingly well developed independent roots, new shoots may develop from adventitious buds at the new root collar belonging to this shoot. This condition occurs frequently.

How long will this state of affairs continue? Evidently not indefinitely, for the root tissue, although more resistant than the stem tissue, slowly succumbs to the attack of the parasite. In the great majority of trees it is already at an end, roots as well as shoots being entirely dead.

And yet the possibilities along this line must not be overlooked. If the resistant substances, whatever they may be, should at any time develop sufficient concentration to obstruct the progress of the fungus—or if, on the other hand, the parasite should lose some of its virulence—a way out of the present difficulty appears. Collins (3) already has noted a case where sprouts successfully threw off the invading fungus for at least seven years, apparently occluding the original lesion. We should be on the lookout for similar cases.

In the meantime we must rejoice in the fact that the chestnut has already revealed, in another direction, an asset of tremendous value. I refer to the observation, already mentioned, that some of these shoots are bearing catkins and mature nuts.

The power of sexual reproduction through seed, borne on these basal shoots, should enable the species to postpone its possible extinction still farther. Since such seed might contain valuable qualities, it should be carefully gathered and planted.

SUMMARY

1. Comparative inoculations on roots and shoots of the American chestnut have demonstrated that the root tissues, including the region of the root collar, are far more resistant to the growth of *Endothia parasitica* than are the stem tissues. In view of previous work indicating an inhibitory effect of tannin on the growth of fungi, it is suggested that the greater resistance of the root tissues may be due, at least in part, to their greater content of tannin compounds or of substances associated with tannin.

2. The greater resistance of the root and root collar tissues is obviously the cause of repeated generations of sprouts from blighted trees. The shoots develop from adventitious buds in the region of the root collar, utilizing at first the root of the old tree, but in time developing their own roots. Ordinarily these shoots die of the blight, but others may have appeared in the meantime, and in this way a succession of "crops" is developed. A further fact of great significance is that many of these shoots have already been found maturing nuts.

These two conditions, namely, the continued development of shoots, and the bearing of seed by these shoots, are most valuable assets to the chestnut, since they may extend the lease of life of the species. In the meantime, variations of parasite or host, favorable to the latter, may occur. Seed of these shoots should be carefully gathered and planted.

BROOKLYN BOTANIC GARDEN,
BROOKLYN, NEW YORK.

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SPECIES OF NECTRIA, GIBBERELLA, FUSARIUM, CYLINDROCARPON AND RAMULARIA OCCURRING ON THE BARK OF PYRUS SPP. IN OREGON

S. M. ZELLER

In connection with a study of European canker of pomaceous fruit trees,[1] the writer has collected or received for identification for several years occasional lots of material suspected as the ascogenous or conidial form of *Nectria galligena* Bres. or closely related species. It was thought well to list, with some notes, this group of species of Ascomycetes and Fungi Imperfecti which are closely linked in the taxonomic literature. The identification of most of the species has been verified by Dr. H. W. Wollenweber, and one new species described by him is listed.

NECTRIA

1. *Nectria cinnabarina* (Tode) Fr.

This fungus is found to a considerable extent on pear twigs which have died back from various causes. After this start in a saprophytic condition, it becomes a means of considerable destruction in living bark, which, although temporarily somewhat devitalized by low temperatures, would doubtless otherwise regain its vigor. *N. cinnabarina* also infects cankered areas on larger branches, especially attacking the spongy parenchyma surrounding lesions produced by the woolly aphis, *Eriosoma pyricola.*

2. *Nectria galligena* Bres.

This species has already been reported[2] as the cause of canker of apple and pear on the Pacific Slope. The control of this canker and its peculiarities as found in America have been fully discussed by the writer in a monographic study.[1] The stage of this fungus represented by *Cylindrocarpon mali* (All.) Wr. is more often found than the ascogenous stage. Pear trees are more severely damaged in the western coastal orchard districts than are the apple trees, because the apples are often protected by the summer application of bordeaux mixture (4–4–50) for the control of apple-tree anthracnose (*Neofabraea malicorticis*).

[1] Zeller, S. M. The European canker of pomaceous fruit trees. Ore. Agr. Exp. Sta. Bul. 222. 1926.

[2] Zeller, S. M., and C. E. Owens. European canker on the Pacific Slope. Phytopathology 11: 464–468. 1921.

3. *Nectria sanguinea* (Sibth.) Fr.

This is the most common species of the section Willkommiotes Wr. which grows saprophytically on a wide range of woody hosts in Oregon. It has been found on apple bark only once. In this case the macroconidia (*Cylindrocarpon*), which were found in large quantities in the crevices of the bark surrounding the sporodochia, were conjugating as shown in figure 2. Some comparatively long conjugating tubes were observed, connecting cells of one spore with those of another. In all cases the nucleus had migrated

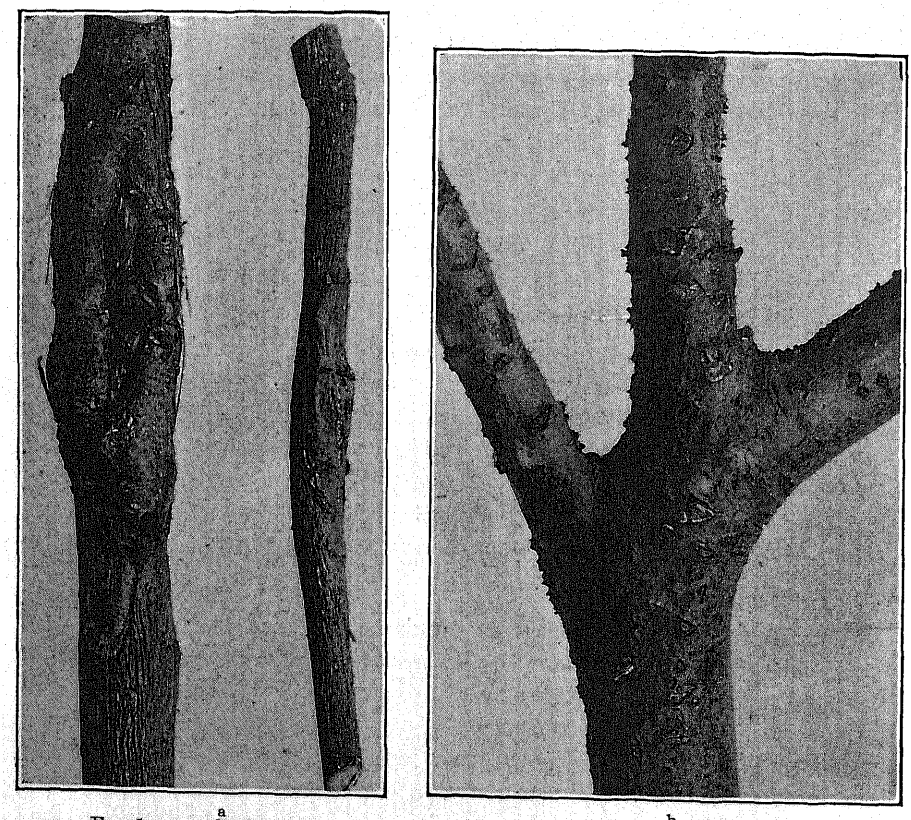


FIG. 1.—a. Cankers of apple invaded by *Gibberella baccata* (Wallr.) Sacc.
b. Sporodochia of *Fusarium oxysporum* Schlecht., breaking through the epidermis of apple bark.

from one cell to the other, producing a binucleate condition previously reported by the writer and Cayley[3] as a condition not uncommonly found in *Nectria galligena* Bres. Another conjugation of cells occurs between two cells of the same spore. This has somewhat the appearance of clamp-con-

[3] Cayley, Dorothy M. Some observations on the life history of *Nectria galligena* Bres. Ann. Bot. 35: 79–92. 1921.

nections in Basidiomycetes but results in more of an open medallion appearance. In these cases of conjugation of cells, the binucleate condition results.

Fusarium avenaceum (Fr.) Sacc. was found associated with the Cylindrocarpon stage of *Nectria sanguinea* on apple bark.

GIBBERELLA

4. *Giberella baccata* (Wallr.) Sacc.

The conidial stage (*Fusarium lateritium* Nees) was found fruiting on small cankers sent to the writer from Cascade Locks, Oregon. These are illustrated in figure 1, a. It would appear that the infection starts at leaf scars, in the buds of small twigs, or in lesions produced by the woolly aphis. These enlarge, producing the open type of canker illustrated. Woolly aphids have undoubtedly stimulated the callus formation in some cases. The sporodochia break through the epidermis of the bark as brick-red pustules about one mm. in diameter. In the illustration these are seen as black spots on the callus tissue.

5. *Gibberella moricola* (Ces. & Not.) Sacc.

G. moricola was found growing saprophytically on reddish, sun scalded bark of apple branches. The fungus was limited to a small area of the blistered epidermis. The material shows both the perithecia and the conidial form, *Fusarium urticacearum* (Cda.) Sacc.

FUSARIUM

6. *Fusarium acuminatum* Ell. and Ev. em. Wr.

Found growing saprophytically on die-back branches of Anjou pear and following woolly aphis injury on apple in the vicinity of Corvallis. Also found on apple bark from Walla Walla, Washington.

7. *Fusarium bulbigenum* Cooke and Harkn.

Under winter injured apple bark, Corvallis. May.

8. *Fusarium graminum* Cda.

Professor H. P. Barss collected this species on bark from an apple canker at the base of the trunk of a small tree, Clatsop County, August, 1922.

9. *Fusarium herbarum* (Cda.) Fr.

On pear bark, Grants Pass and Colton. April and June.

10. *Fusarium oxysporum* Schlecht.

Two young apple trees which had suffered apparent winter injury were completely covered above the main crotch, with the sporodochia bursting through the epidermis as illustrated in figure 1, b. Branches were infected for distances of 4 or 5 feet. Material was sent to Dr. Wollenweber who

states that this is the first report of the occurrence of *F. oxysporum* on apple trees. His notes on the cultural characters of this strain from apple bark follow:

"The fungus developed terminal chlamydospores and all other types of this resting stage in abundance in pure culture. . . . It develops large but few sporodochia covered by ochraceous to pale salmon-colored conidial masses. Blue sclerotia or sclerotial plectenchyma of medium to large size up to 3 mm. occur on potato tuber plugs. On rice the color is lilac rosy, turning blue by addition of sufficient alkali. The conidia have the shape of *F. oxysporum* Schlecht., and at any rate, if it is not identical with this species, it is not far off."

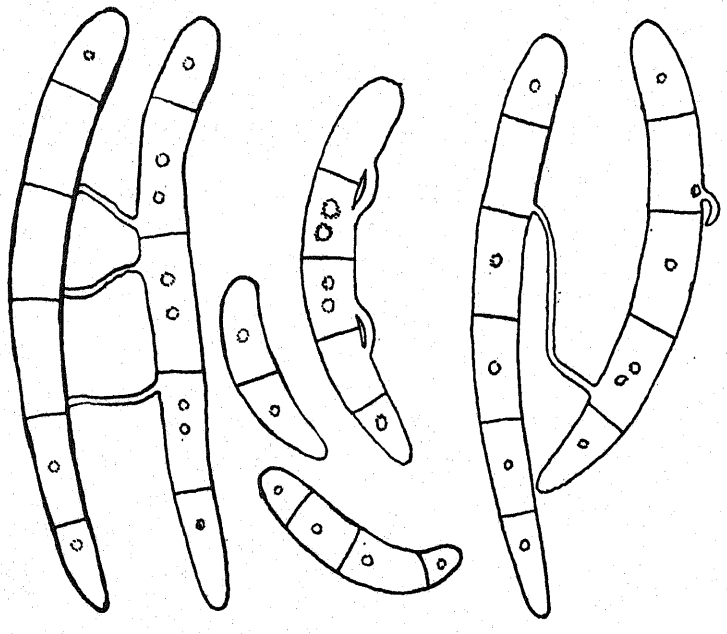


FIG. 2. Macroconidia of *Nectria sanguinea* (Sibth.) Fries, showing the peculiar conjugation of cells when these spores are lodged in masses in the crevices of bark. ×1,000.

The collections were made by the writer and F. M. Green at Hood River, November, 1922, and again in Corvallis, July, 1925.

11. *Fusarium polymorphum* Matr.

On pear bark, Grants Pass, April 24, 1924. Found to be associated with *F. herbarum* and *Ramularia obtusispora* in the same piece of bark.

Although not reported from Oregon, *Fusarium equiseti* (Cda.) Sacc. was received from San Jose, California, on pear bark.

CYLINDROCARPON

Besides the species of *Cylindrocarpon* which are definitely known to represent the macroconidial forms of species of *Nectria,* one other species has been found in Oregon. This species proved to be undescribed and the diagnosis by Dr. Wollenweber appears below.

12. **Cylindrocarpon angustum** Wollenweber sp. nov.

Sporodochiis tuberculariibus, ad basim plectenchymicis, plus minusve erumpentibus, isabellinis vel incarnatis; conidiis cylindratis, rectis vel leniter curvatis, utrinque obtusis, 5-septatis, 57–81 × 3–4 micr., rarioribus 3-, 4-, 6-, rarissime 7-septatis; 6–7-septatis, 69–92 × 3.5–4 micr. Chlamydospora non visa sunt.

Habitat: In rimis corticis Piri mali, Clatsop County, Oregon. Amer. borealis, autumnos 1922 leg. H. P. Barss (in Ore. Agr. Col. Herb. no. 4849 et Wollenweber Herb.).

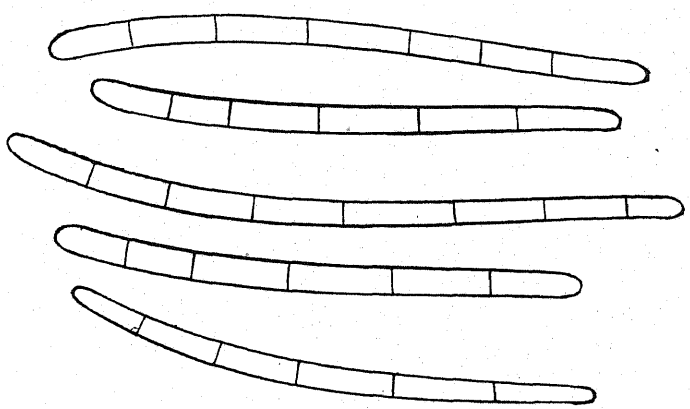


FIG. 3. Macroconidia of *Cylindrocarpon angustum* Wollenw., n. sp. × 1,000.

Figure 3 illustrates the outline of the macroconidia (× 1,000) of *C. angustum.* The macronidia are strikingly smaller in diameter than those of congeneric species.

RAMULARIA

13. *Ramularia obtusispora* (Cke. and Hark.) Wr.

On pear bark, Forest Grove and Grants Pass, April and May. As mentioned above the collection of this species found at Grants Pass was associated with *Fusarium polymorphum* and *F. herbarum.*

OREGON EXPERIMENT STATION,
CORVALLIS, OREGON.

EFFECTS OF WHEAT TREATED WITH COPPER CARBONATE UPON THE COMMON HOUSE MOUSE (*MUS MUSCULUS*)

W. W. MACKIE[1] AND FRED N. BRIGGS[2]

Copper carbonate dust applied to seed wheat for bunt control has been found to prevent insect attack in stored grain,[3] and it has been repeatedly observed by the writers that seed wheat thus treated was not attacked by mice. In order to determine whether copper carbonate dust is poisonous to mice or acts solely as a repellant, a series of experiments were conducted in which wild mice were fed in strict confinement.

On August 4, 1925, two adult and five half grown mice were trapped in the agronomy storage room. These were placed in cages 12 inches square. They were confined for one month on a pure wheat diet before the experiments with treated wheat were started. All of the young mice soon developed rickets and two of them were killed because of the severity of the attack. On the addition of a few drops of cod liver oil to the diet each week, the disease soon disappeared and did not occur again. Later one adult mouse escaped, leaving four mice with which to start the experiments. The amount of wheat eaten by the various mice was carefully weighed for a period of one week. The adult mouse ate approximately 5.0 grams of wheat daily and the others about 3.5 grams each per day.

Each mouse was kept in a separate cage and was given clean bedding once each week. The cages were numbered I, II, III, and IV; the adult mouse occupying number III. Beginning September 4, 1925, numbers III and IV were given wheat treated with copper carbonate at the rate of one ounce per bushel, while numbers I and II served as checks. The treated wheat was eaten just as readily as the untreated wheat and approximately in the same quantities without any apparent ill effects at the end of the seven-day period.

All the mice were then put on the untreated wheat ration for a week. Thereafter the mice in cages III and IV were fed wheat treated with copper carbonate at the rate of two ounces per bushel, while numbers I and II again served as checks. Later, numbers III and IV were fed for a week on

[1] Associate Agronomist, in charge of Cereal Investigations, California Agricultural Experiment Station.

[2] Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[3] Mackie, W. W. Prevention of insect attack on stored grain. Calif. Agr. Exp. Sta. Circ. 282. 1–8. 1925.

wheat treated with copper carbonate at the rate of four ounces per bushel. In both instances they ate the wheat in normal amounts, without apparently suffering any ill effects.

After another week of a ration of untreated wheat, all four mice were fed for 15 days with wheat treated with copper carbonate at the rate of four ounces per bushel, without ill effects.

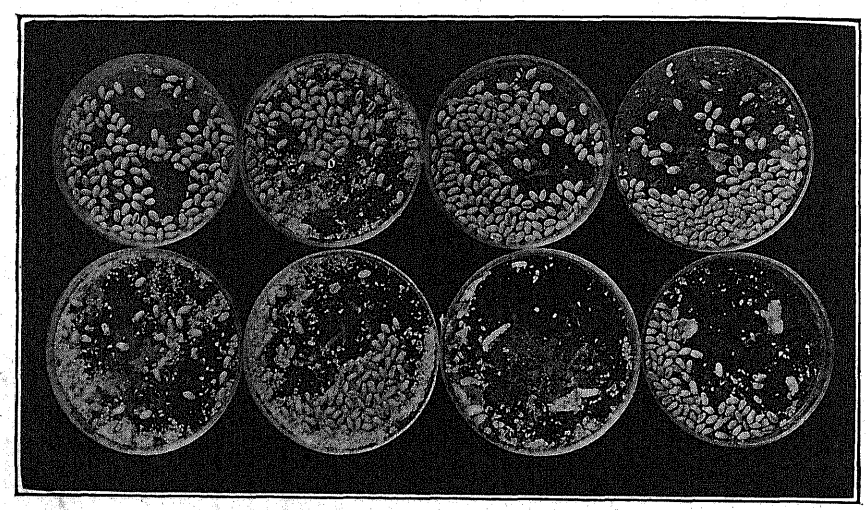


FIG. 1. The petri dishes in the top row contain wheat treated with copper carbonate dust at the rate of one ounce per bushel. Those in the bottom row contain untreated wheat. Each dish originally contained 10 grams of wheat, and the photograph was made after 48 hours of feeding. Only the treated seed in dish No. 2 has been eaten appreciably.

When it was apparent that the mice would not be readily killed by eating treated wheat, another experiment was started in order to determine if they would choose between treated and untreated wheat. Two dishes of wheat were placed in each cage, one containing wheat treated with copper carbonate at the rate of one ounce per bushel and one with untreated wheat. Figure 1 shows the dishes with the wheat remaining after two days. Number II was the only mouse that ate the treated wheat to any extent. The experiment was repeated with wheat dusted with two ounces of copper carbonate per bushel. Following this treatment none of the mice ate the treated wheat (Fig. 2).

DISCUSSION

Mackie[4] has shown that wheat treated with copper carbonate dust is free from weevil injury as contact with the dust kills the weevil. Hoffman and

[4] Mackie, W. W. *Loc. cit.*

Belton[5] state that "the treated grain is poisonous to live stock of all kinds as well as to rodents." However, they do not give any data on this subject. There is no doubt that copper carbonate is poisonous and therefore should be handled with some care. Data presented here indicate that mice do not get enough copper when fed in the copper carbonate form to injure them when fed only treated wheat, even when the rate of treatment is four ounces per bushel or twice as much as ordinarily is used on the farm. If a mouse

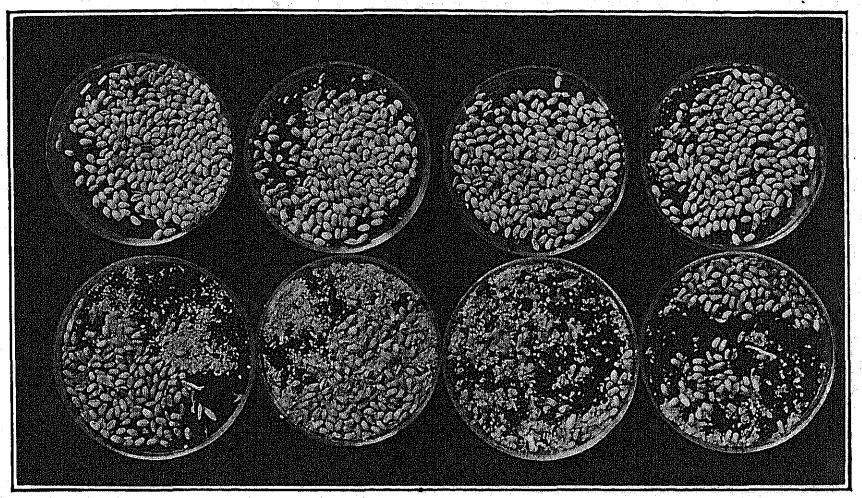


FIG. 2. The dishes in the top row contained wheat treated with copper carbonate at the rate of two ounces per bushel. The dishes in the bottom row contain untreated wheat. Each dish originally contained 10 grams and was photographed after 48 hours of feeding. The treated seed is scarcely touched.

which consumes five grams of wheat per day ate all of the copper carbonate on the wheat, he would consume only .0208 grams per day; but a mouse leaves most of the chaff in eating wheat and in so doing avoids most of the copper carbonate.

SUMMARY

Copper carbonate causes no apparent injury to mice when consumed by them on wheat as ordinarily treated to control bunt.

When untreated wheat is available mice will avoid wheat treated with copper carbonate.

So far as the effect upon mice is concerned, copper carbonate is a repellant and not a poison.

[5] Hoffman, A. H., and H. H. Belton. Machines for coating seed wheat with copper carbonate dust. Calif. Agr. Exp. Sta. Bul. 391. 1–16. 1925.

An adult mouse may consume approximately 1/3 of a pound of wheat per month and a half-grown mouse about 1/4 of a pound per month.

Because of the mouse-repellant action of copper carbonate dust, treated seed wheat may be stored exposed to mice for indefinite periods awaiting its final use. Under ordinary farm conditions, this action of copper carbonate dust will save farmers great quantities of valuable seed.

INJURY TO GLASSHOUSE PLANTS FROM HYDROCYANIC ACID GAS, FOLLOWING THE APPLICATION OF COPPER FUNGICIDES

E. F. GUBA

It is known among citrus growers in California and Florida that the fumigation of citrus trees with hydrocyanic acid gas following the application of bordeaux mixture is accompanied by serious injury to the trees. The injury is manifested in the form of browning of the leaves and defoliation, often accompanied by severe pitting of the fruit. In some cases injury has resulted as late as 11 months after the copper spray was applied, although ordinarily little if any injury occurs six months after spraying. To avoid this injury where brown rot and other diseases are to be controlled, some growers have adopted the practice of spraying two or three days after fumigation, while others have discontinued the use of bordeaux entirely in favor of other fungicidal preparations free of copper.[1]

The writer is not aware of any literature, popular or otherwise, offering any objections to the use of copper fungicides in greenhouses. However, many greenhouse men, not knowing of the incompatability of copper fungicides and hydrocyanic acid gas, used in the order given, have lost entire houses of tomatoes and cucumbers in the very height of picking. In the majority of cases the injury has been attributed to overdosage of cyanide, over-exposure, or other conditions under which fumigation took place. Rarely has injury been associated with the presence of copper fungicides on the plants.

Hydrocyanic acid gas is a very effective fumigant for killing certain greenhouse insects. It is obtained from the action between sodium or potassium cyanide and sulfuric acid, or between calcium cyanide and water. With the presence of copper fungicides on the foliage, the hydrocyanic acid gas liberated combines with the copper to form cupric cyanide $Cu(CN)_2$, a very poisonous and unstable compound giving off cyanogen and leaving cuprous cyanide $Cu(CN)$.[2,3] In trials conducted in the greenhouse on cucumber and tomato plants treated with bordeaux, pyrox and copper lime

[1] WOGLUM, R. S. Fumigation of citrus trees for control of insect pests. U. S. D. A. Farmers' Bulletin 1321. 1923.

[2] TURNER, F. M., et al. The condensed chemical dictionary. The Chemical Catalog Co., Inc., N. Y. 525 pp. 1919.

[3] BLOXAM, C. L. Chemistry, inorganic and organic with experiments. 11th ed. rev. P. Blakiston's Son and Co., Philadelphia. 1923.

dust, all plants were injured equally following fumigation with hydrocyanic acid gas, while plants not treated with copper fungicides but exposed to the fumigant were unaffected. No data have been obtained to indicate how long after the application of copper the houses may be fumigated without danger of injuring the plants, although we have record of an instance of no injury from fumigation with calcium cyanide 46 days after the copper treatment.

Since hydrocyanic acid gas is used by the majority of growers for the control of certain insects affecting greenhouse plants, it is very essential that any recommendations for the control of fungous diseases involving the use of bordeaux mixture or copper lime dust should be accompanied by a word of warning. Where fungous diseases are of sufficient economic importance in the greenhouse to warrant the use of copper fungicides, the cyanide fumigant should always precede the application of the copper by one or two days, or the use of cyanide should be abandoned in favor of tobacco fumigants. Under any conditions cyanide fumigation should never immediately follow the use of copper.

DEPARTMENT OF BOTANY,
MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION

NEGATIVE RESULTS OBTAINED BY THE USE OF PARADICHLOROBENZENE AGAINST THE ROOT-NEMATODE, HETERODERA RADICICOLA

H. R. Rosen

The successful control of peach tree borers obtained by applying paradichlorobenzene to the soil is now well known. Though this remedy is of comparatively recent development, it has very largely, if not entirely, replaced the old laborious digging-out method. The chemical is easily applied and costs but a few cents a tree.

In view of these facts, and in view of the killing of the insect by the gas liberated in the soil, it seemed desirable to ascertain the action of the same chemical against another soil-inhabiting metazoan, the common root-nematode, *Heterodera radicicola*. Two lots of sandy soil were obtained, one in which the nematodes had been so abundant as to completely prevent the development of marketable sweet potato roots, and the other one in which tomato roots had been severely infested. Both soils had been under close observation by the writer, and the effects on the roots were noted during the growing season of 1925 up until October. In each instance the soil gathered represented portions in which infested roots were common. Sufficient soil of each type was obtained on November 17, 1925, to fill three pots, each 6 inches in diameter. Three pots, two of soil in which the tomatoes had grown, and one in which the sweet potatoes had grown, received 0.25 ounces of paradichlorobenzene per pot. The chemical was applied in the center of the pot, about 2 inches below the surface. The other three pots, untreated, served as checks. Tomato seed, of a wilt-resistant Stone variety, were then thickly sown in each pot.

As germination proceeded, it was quite evident that the chemical had injured a fairly large number of the seedlings, especially those near the chemical, since all the treated pots had a much lower stand toward the center and the seedlings were considerably retarded. When some of these were dug up it was noticed that roots and lower parts of stems were discolored, shriveled, and, in a number of instances, completely killed. An enlargement or swelling was often noted in the parts adjoining the discolored, dead areas. Microscopic examination of recently injured parts showed no fungus or bacterium which was common to all, and as the untreated pots all possessed seedlings with no comparable symptoms, there can be little doubt that the chemical was responsible for the damage. However, quite a few

seedlings remained standing in the treated pots, and full opportunity was afforded to note the effects of the chemical on the nematode. The roots of untreated plants were severely infested, and the roots of the treated plants, although not having as many root-knots, also were infested. As the plants grew larger, root-knots on treated plants became more and more common, although they apparently were not quite as abundant as in the untreated. Irrespective of this, however, every one of the treated plants—and over 100 of these were examined—showed some root-knots. Microscopic examination of the swellings all showed one or more nematodes in the affected parts.

As there is a possibility of lack of gas diffusion in a moist soil, the 6 pots were permitted to dry out in the greenhouse for about two months, and the soil reworked and then again seeded to tomatoes. This drying killed most if not all the nematodes in the untreated pots, and the few swellings which were definitely identified as nematode injury all appeared in the treated pots. The characteristic odor of paradichlorobenzene which was noticed at the second seeding, although not nearly as strong as when first applied, indicated that the generated gas had sufficient opportunity of acting on the nematodes. The pots were carefully handled throughout, and efforts were made to prevent reinfestation. In view of these results, one may conclude that paradichlorobenzene is not an effective remedy against the nematode, *Heterodera radicicola*. The writer is greatly indebted to the Hooker Electrochemical Company for supplying the chemical used in the trials.

While negative results of this sort are of little value, they are offered with the hope of eliminating unsuccessful efforts of other workers.

UNIVERSITY OF ARKANSAS,
FAYETTEVILLE, ARK.

BOOK REVIEW

Plant Disease Fungi. By F. L. Stevens, Ph.D., Professor of Plant Pathology, University of Illinois. 469 pp. Illus. The Macmillan Company, New York, 1925.

This book follows the "Diseases of Economic Plants" (1910 and 1922) and "The Fungi Which Cause Plant Disease" (1913) by the same author. As stated in the preface, its aim "is to present the more important facts concerning the morphology and taxonomy of the fungous parasites that affect plants of importance in the continental United States with some discussion also of the more significant facts of morbid histology." To accomplish such an end a book must have both substance and form—must contain an adequate body of well chosen and accurate information and must present this in a telling and easily accessible manner. These requirements are fulfilled by this volume with an effectiveness that should render it as successful as its two predecessors. The form of the book is good. It is well turned out, clearly printed on a good quality of paper, contains an abundance of helpful illustrations, and all in all presents an attractive appearance. The arrangement of the subject matter is logical and easily followed. The fungi considered are treated in separate chapters by groups, and of these groups the main representatives are characterized. Their effects and life histories are touched upon briefly.

Of the 469 pages, the space and emphasis allotted to each of the main groups of fungi are justly proportionate to their importance. After a brief introduction, the Myxomycetes as Division 1 are dealt with in six pages; the Schizomycetes as Division 2 receive 41 pages; while the consideration of the Eumycetes as Division 3 occupies the bulk of the book. Under the Eumycetes, a general discussion of 6 pages leads to a detailed consideration of the Phycomycetes, 31 pages; the Ascomycetes, 123; the Basidiomycetes, 121; and the Fungi Imperfecti, 110 pages. A helpful index with a purely alphabetical arrangement that mingles authors, subjects, diseases, hosts, and parasites completes the total.

As the subject matter is to some extent a reconsideration of that already included in the two previous books, a greater freedom from typographical errors justifiably might be expected. Apparently a careful scrutiny of the book would yield a considerable list of such errors which, however, do not seem to require enumeration here; as, even though they have escaped thus far, one assumes that they will be corrected. Moreover, such an enumeration of typographical errors seems all too often to display the zeal of the reviewer rather than to offer belated aid in the onerous task of revision and correction. Meanwhile, those to whom these errors are self evident will not find the considerable value of the book reduced thereby; while for those by whom they are unnoted, they do not, pragmatically, exist.

Here and there throughout the subject matter occur statements that are somewhat misleading. For example, the summary dismissal of *Physoderma zeae-maydis* on page 61 as "discovered in India by Shaw in 1910 and in Illinois by Barrett in 1911" might be prevented from giving an inadequate impression of the history and occurrence of this species by the addition of a brief reference to the thorough investigation of its distribution in the southern part of the corn belt of the United States by the Plant Disease Survey. Also, in the discussion of the genus *Bremia* on page 79, the statement that

"There is only one species" hardly does justice to *B. graminicola*, established in 1913 by Naoumoff, and *B. elliptica*, *B. microspora*, *B. ovata*, *B. saussureae*, and *B. sonchi*, adequately described in 1914 by Sawada. Nor does the discussion of *Peronospora parasitica* on page 80 give due consideration to the fundamental work of Gaumann in justifiably separating this aggregate into several well defined species in 1915. Moreover, it is perhaps with a certain inconsistency that the Actinomycetales are placed among the Bacteria, when the description of the genus on page 48 emphasizes "a much branched mycelium which may break up into segments that function as conidia"; and the discussion of *Actinomyces scabies*, describes "Spores more or less cylindrical, 0.8–0.9 x 1.3–1.5 μ, from dextrorse, spiral hyphae of 3–14 turns," following Dreschler, whose intensive investigation of the genus has demonstrated convincingly that the Actinomycetales should be classed among the Hyphomycetes rather than among the Bacteria. Though not misleading, sentences such as "none are known among the pathogenes" (page 11), and "There is one or more carpogonia" (page 89), may be somewhat distressing; while the statement (page 332) that "Pycnidia with very small spores are sometimes called spermagonia, especially if the spores do not germinate, a custom to be deprecated" as it now stands leaves one wondering if the custom, at least in pathogenic fungi, should not rather be commended or even encouraged—perhaps with bordeaux mixture.

The numerous figures aid materially in the presentation of the subject matter; as they are for the most part well chosen, and well reproduced, to illustrate the descriptions that they accompany. In some cases, however, it is a question whether the figures used are as effective as others that might have been selected. Figure 37, showing the effect of potato scab on the phellogen, for example, might be replaced advantageously by drawings of the causal organism itself. Figure 69, though historic as Millardet's own, would be more instructive perhaps if taken from the beautiful plates of Istvanffi and Palinkas. Also, certain illustrations have lost much in being reproduced, as for example, figure 47 of *Physoderma zeae-maydis*, figure 56 of *Phytophora phaseoli*, 154 of *Herpotrichia nigra*, 336 of *Myxosporium corticolum* and others. The title of figure 74, reading "A sporangium with a columella (Mucor)," is rather misleading, as the most obvious part of the figure shows "mycelium (3 days old) of *Phycomyces nitens* grown in a drop of gelatine with a decoction of plums" as stated in the legend under the original figure in Sachs.

In conclusion, the outstanding fact that the book is a very valuable tool for the mycologist and plant pathologist, again should be emphasized. Therefore, having done my duty as a critic in noting certain scratches in the finish of this tool, I shall now proceed with the complacency of all critics, to make good use of it in my own work and that of my classes.—W. H. WESTON, JR.

ABSTRACTS OF PAPERS READ AT THE MEETING OF THE SOUTHERN SECTION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY WITH THE TWENTY-SEVENTH ANNUAL CONVENTION OF THE SOUTHERN AGRICULTURAL WORKERS, AT ATLANTA, GEORGIA, FEBRUARY 3–5, 1926

Results of five years' selection for freedom from internal cob-discoloration in corn.—R. A. JEHLE, F. W. OLDENBERG, and C. E. TEMPLE.

A series of tests was conducted for a period of five years to determine the relation of the pink, gray, brown and other discolorations of the vascular and surrounding tissues of the interior of the cob to yield and to the root, ear, and stalk rot diseases. In each test the best seed ears were selected and the butts and tips cut off so that the interior of the cob could be examined. The corn was divided into three or more lots, according to the amount and intensity of internal cob-discoloration. At least two lots, one showing little or no internal cob-discoloration and one showing marked internal cob-discoloration, were planted in each test. The following results were obtained.

1. In 116 tests with field corn the average yield from cobs with little or no internal cob-discoloration was 60.11 bu. shelled corn per acre and the yield from cobs with marked internal cob-discoloration was 51.75 bu. shelled corn per acre. Thus the yield from cobs with little or no internal cob-discoloration was greater than the yield from cobs with marked internal cob-discoloration by 8.54 bu. or 16.56 per cent.

2. This increase on poorer corn land was 9.15 bu. per acre or 19.26 per cent, and on the best corn land it was only 4.75 bu. or 6.1 per cent. Thus the difference in yield was greater on the poorer corn land.

3. The difference in yield also was found to vary with the intensity of internal cob-discoloration.

4. Internal cob-discoloration can be decreased by selection. It has been almost entirely eliminated in four to five years by planting only seed of ears free from internal cob-discoloration.

5. Corn grown from ears with the least internal cob-discoloration has a more uniform stand and a smaller number of fallen and broken stalks, and less prematurely killed plants than corn grown from ears with the greatest amount of internal cob-discoloration.

Brown spot disease of soybean.—F. A. WOLF and S. G. LEHMAN.

Brown spot is primarily a foliage disease but occurs also on stems and pods. It first appears as irregular dark brown patches upon the cotyledons. Infections next appear on both surfaces of the unifoliate leaves as angular, reddish brown lesions varying in size from 1 to 5 mm. A pale green or chlorotic zone surrounds the diseased areas. The disease spreads to the trifoliate leaves where lesions become very numerous and coalesce to form large, light-brown, irregular areas in which the pigmentation gradually deepens to a dark brown or blackish brown color. As the disease progresses the lesions become so numerous as practically to cover the leaf area completely. In seasons favorable for

the disease, considerable defoliation of affected plants occurs. Defoliation begins with the lowermost leaves, which are first to become infected, and progresses upward with the advance of the disease on the plant.

On the stems the disease appears as indefinitely margined, brown discolorations varying in size from mere specks to areas several centimeters in length. Similar discolored areas occur on petioles and pods.

Globose pycnidia possessing thin, membranaceous walls and opening by a large pore are embedded in the tissues bearing mature lesions. The pycnidia are filled with hyaline, filiform, curved, indistinctly septate conidia. Inoculation experiments have demonstrated the pathogenicity of the causal organism. This fungus is identical with *Septoria glycines* described by Hemmi as the cause of a wide-spread and severe epiphytotic of brown spot disease in Japan in 1914. No ascogenous stage has been found. The fungus overwinters in the conidial stage on diseased leaves and stems and very probably survives in seed from diseased plants.

Soybean anthracnose.—S. G. LEHMAN and F. A. WOLF.

Soybean anthracnose was first observed at Raleigh, North Carolina, in 1920, and has reappeared repeatedly since then. The disease occurs on stems and pods but has never been observed on leaves, although plants in all stages of development are subject to attack. The disease reaches its most destructive stage at the time the pods are maturing. Diseased plants die prematurely, and affected portions become thickly covered with black acervuli which bear numerous setae. Pure cultures of the fungus have been isolated from cotyledonary tissues of seed from diseased pods. Both conidial and ascogenous stages of the causal fungus have been studied.

The soybean fungus was at first thought to be identical with the apple bitter rot fungus, *Glomerella cingulata*, but cultural and morphological comparisons have led to the conclusion that the two are distinct. Asci, ascospores, and conidia of the former are larger than those of the latter. The two fungi differ in appearance when grown on agar media and produce different types of rot on apple fruits. Conidia and ascospores of the soybean organism readily cause infection when sprayed on soybean plants; but, under the same conditions, spores of the apple bitter rot fungus fail to infect soybeans, nor does infection occur when bits of mycelium of the bitter rot fungus are inserted into soybean stem tissues. The fungus overwinters in diseased seed and on diseased stems and pods.

It is believed that the soybean anthracnose fungus studied by the writers is identical with *Colletotrichum glycines* Hori, which causes an anthracnose of soybean in the Orient. Since the perithecial stage is described by the writers for the first time, the soybean anthracnose fungus is given the new name, *Glomerella glycines* (Hori).

Pythium root rot of soybean.—S. G. LEHMAN and F. A. WOLF.

In July, 1923, soybean plants in the breeding grounds of the North Carolina Agricultural Experiment Station became affected with a root rot disease which obviously was different from other root rot diseases then known on this host. The presence of the disease was first made evident by the appearance of groups of withered and dying plants scattered throughout the field. On closer examination it was found that these plants were being attacked by an organism which was causing a decay of the roots and lower stem portions. By appropriate methods a fungus was isolated which is referred to as *Pythium de baryanum*. The fungus apparently enters the plants at or near the soil level, whence it grows downward into the roots and upward into the stem to a height of

several inches. Newly invaded stem tissues are at first watery and translucent, but as decay progresses the lesions become brown and the cortical tissues may become so completely disintegrated as to fall away. When diseased plants are pulled from the soil, the disintegrated cortical tissues often remain in the soil, leaving the central woody cylinder exposed.

The causal fungus is described and its taxonomic position indicated. The pathogenicity of the causal fungus was demonstrated by infection experiments.

Work of the Office of Cotton, Truck and Forage Crop Disease Investigations of the Bureau of Plant Industry, United States Department of Agriculture, in relation to Southern agriculture.—H. A. EDSON.

The field assigned by the Bureau of Plant Industry, United States Department of Agriculture, to the unit officially designated "The Office of Cotton, Truck and Forage Crop Disease Investigations" is the study of the cause and control of diseases of cotton, potatoes, sweet potatoes, truck crops, forage crops and herbaceous ornamental plants as well as drug and related plants. The active projects of the office cover only a portion of this field since some of the lines indicated have never been specifically financed in the budget and the funds made available for several others are so limited that only incidental work on them is possible. For this reason projects on the diseases of cotton, ornamentals, drug plants and many of the minor vegetable crops are inactive. In the prosecution of projects for which funds are available, it is the policy to select problems which are national, or at least broadly regional, in scope but which cannot be solved readily without crossing state lines. Cooperation with state stations in the investigation of the local phases of all problems is courted as a matter of policy and arranged for whenever feasible. Among the active projects of special interest to Southern agriculture may be mentioned the following: virus diseases of potatoes; sweet potato diseases; black heart of celery; mosaic disease of lettuce and tomatoes; lettuce breeding for resistance to brown patch and downy mildew; nailhead rust of tomatoes; tomato breeding for resistance to Fusarium wilt, nailhead rust, and mosaic; cabbage breeding for resistance to yellows; bean breeding for resistance to bacterial blight and mosaic; transit diseases of snap beans; watermelon diseases; alfalfa diseases; clover diseases.

Further experiments in the control of watermelon anthracnose with dusts and sprays.—O. C. BOYD.

The project begun at the Thomasville station in 1924 for the control of watermelon anthracnose was continued in 1925. Again Bordeaux mixture, a home-mixed copper-lime dust, and a commercial copper-lime dust were used on the Watson variety. The spray formula used in 1925 was 4–8–50, the "8" representing hydrated lime, with Kayso added in the last three applications. Both dusts contained 20 per cent monohydrated copper sulphate, and were applied to the vines with a hand duster equipped with a long outlet pipe. All dust applications were made in the late afternoon after the wind had subsided—not in the early morning on damp vines as in 1924. The spray applications were made with the same machine that was used the previous year, *i.e.*, the Hardie "Magic," an engine-power truck sprayer with lead-hoses, bamboo rods, and nozzles. Six applications of each fungicide were made with intervals of from seven to ten days.

The experimental field comprised 3.6 acres, with three plats of each treatment and three of the controls. Each dusted plat contained three rows, and the sprayed and control plats contained four rows each.

Anthracnose was the only disease of any consequence in the experiment. It was first observed on May 25 in a single hill of a dusted plat. The disease spread steadily in the untreated plats, first showing there about June 1. On June 20, the date of the fifth application of fungicides and four days prior to the first picking, practically every hill in the control plats showed leaf spots, with the majority of the older melons affected with pox. An occasional hill throughout the sprayed and dusted plats also showed both leaf and melon infection. By July 9, the date of the third picking and ten days after the last application, the check plats appeared brown and barren from defoliation and death of vines. The treated vines were still green and growing, but the disease was beginning to spread rapidly due to the exposure of considerable new growth that developed after the last application of fungicides.

There was little doubt but that the sprayed vines retained more fungicide per unit amount of copper applied than the dusted vines, and that the spray deposit was more resistant to washings by rain than the dusted vines. Nevertheless, the dusts, with applications ranging from 15 pounds per acre at the beginning to 80 pounds at the close of the season, furnished satisfactory control even when dusted on dry vines. The spray mixture furnished slightly better control on the leaves, with a doubtful difference on the melons. The number of mature melons per acre harvested under the different treatments, with the percentage of poxed melons in each case, was as follows: checks, 416 with 67.5 per cent; spray, 411 with 20 per cent; home-made dust, 431 with 22.9 per cent; commercial dust, 419 with 25.7 per cent poxed. The market value per acre, less pox, was as follows: $25.57, $77.77, $76.13, and $71.20 for the treatments in the same order as in the preceding sentence.

The amount of each of the dusts used per acre in the six applications was 325 pounds, containing 22.75 pounds of copper, compared with 625 gallons of Bordeaux mixture with 19 pounds of copper. The cost of the different materials was as follows: 100 gallons of spray, about $1.25; 100 pounds of the home-mixed dust, $4.80; 100 pounds of the commercial dust, $10.00. The total cost per acre was: $10.40 for the spray, $17.75 for the home-made dust, and $34.50 for the commercial dust. (The cost of the spray treatment might well be reduced $2.20, the cost of the 11 pounds of Kayso in the last three applications, making the cost $8.20 instead of $10.40.) Hence, the net returns per acre were: $25.57 for the untreated plats, $67.37 for the spray, $58.38 for the home-made dust, and $36.70 for the purchased dust.

Although the dusts this season furnished practically as good control as the spray mixture, with only slight injury to the vines, it should be remembered that anthracnose was not as prevalent as usual and that only hand machines were used for dusting. Before dusting can be given a definite place in anthracnose control, reservations will be made for further experimentation which will involve the use of mule-drawn dusters. It seems that the grower could afford to insure his crop for only about $8.00 per acre by spraying, or perhaps for about $18.00 by dusting with home-mixed dusts. The advisability of dusting with materials costing 10 cents per pound or more might depend on the severity of the disease in question and on the price of melons.—Georgia State Board of Entomology Experiment Station, Thomasville, Ga.

The pecan scab fungus.—J. B. DEMAREE.

The organism which causes the disease of the pecan commonly known as scab has for many years been referred to in literature under the name of *Fusicladium effusum* Winter. The type collection of the fungus was collected by Dr. F. S. Earle on white hickory near Cobden, Illinois, in 1882.

Bonorden described the genus *Fusicladium* as producing its conidia singly or in pairs on short unbranched hyphae. Until recently it was supposed that the pecan scab fungus also produced its conidia in a similar manner. It is now known that this organism produces its conidia in long chains acropetally on either simple or branched conidiophores; characters which are not considered typical of the genus *Fusicladium*. The appearance of the fungus on living pecan tissues and also its reaction on artificial culture media indicates that it has several characters in common with the form-genus *Cladosporium*. Its morphological resemblances to *C. carpophilum* Thum. are very striking and suggest that they may be congenerically related. The complete manuscript gives more comprehensive reasons for transferring the fungus to the genus *Cladosporium*.

The future of the chestnut as an orchard, ornamental and forest tree in the Southern Appalachians.—G. F. GRAVATT.

As the chestnut blight spreads over the Southern Appalachians, the few orchards of that region will be exposed to infection from diseased native growth. With some strains of the hairy Chinese chestnut (*Castanea mollissima*) and the Japanese chestnut (*Castanea japonica*) orchardists can control the blight at a reasonable cost. The cutting out of all infected bark tissue, together with ½ to 1 inch of healthy bark and ½ inch of sapwood under the canker, followed by painting with a mixture of creosote and coal tar, has proved very effective in eradicating the disease. Less drastic treatment in experienced hands is satisfactory.

Successful inoculations on varieties and species of chinquapins from different parts of the Gulf states and Arkansas indicate that the blight will eventually spread over the chinquapin area of the South.

The ornamental American chestnuts of the Southern Appalachians are doomed. Owners who are dependent upon these trees for shade should take immediate steps to plant resistant chestnuts or other kinds of shade trees to replace the native chestnuts when they die. Many strains of the Japanese and hairy Chinese chestnuts are sufficiently resistant to the blight to be grown with hardly any treatment, while others are rather susceptible and require considerable treatment. The beauty of these chestnuts and their production of edible nuts make them desirable trees for many situations.

The chestnut forest growth of the Southern Appalachians is also doomed. The blight is spreading more rapidly in the South than in the North, and already about one-fifth of the chestnut growth has 80 per cent or more of the trees infected.

Owners should make plans to utilize their chestnut poles before they are killed by blight because killed poles will usually not be accepted by purchasers. Severe losses have been suffered by many owners of standing poles, who failed to cut them before they were killed.

The forecast map on future spread, which predicts the death of most of the commercial chestnut of the Southern Appalachians before 1935, forces consideration of the problem of growing tanning materials to replace the chestnut. Over one-half of our present domestic production of tannin is secured from chestnut, and we are already importing one-half of the total tannin supplies consumed by our five hundred million dollar tanning and leather industry. From the viewpoint of the American farmer, the manufacture of chestnut tannin extract and leather is an ideal industry. In addition to providing the workers as a market for his food products, this industry consumes vast quantities of tanning materials from the farmers' wood land and uses his hides.

The investigations of the U. S. Office of Forest Pathology which affect this industry have been directed during the past two seasons toward determining, first, the present

distribution and rate of spread of the blight, and next, the rate of deterioration of killed trees.

The work of locating and breeding resistant chestnuts to replace the American chestnut as a source of tannin is being continued on a small scale.

Report on the experiment of 1925 for pecan scab control at Baconton, Ga.—O. C. BOYD.

The pecan station near Albany, Ga., which was established in the spring of 1925, undertook in that year among other projects that of pecan scab control. By reason of the more suitable orchard conditions, with respect to the prevalence of the disease, susceptible varieties, and other factors, the experiment of the first year was conducted at Baconton, Ga. The object of the experiment was to test the effectiveness of some copper dusts in comparison with Bordeaux mixture. The primary motive was the realization by growers of the difficulties in, and the limitations to, the spraying of pecans, and a mere hope that there might be a dusting method that might supplement to advantage, or possibly even replace spraying. It is well known now that dusting is proving both effective and practicable in the prevention of diseases of other fruits, and if such a method should prove effective and economical in preventing scab in the less susceptible varieties of pecans, such as Alley, Schley, Pabst, and others, it would mean thousands of dollars to growers and to the pecan industry.

The experiment was conducted on 16-year old Delmas, Schley, and Alley trees, the majority being Delmas. The Delmas variety began to scab in 1919, and the other varieties in 1922 or 1923. The 1924 crop of Delmas was almost a total loss from scab. The orchard equipment consisted of a Dosch fruit duster and sprayers of the Hardy and Friend makes. The fungicides compared were: Bordeaux mixture 4–8–50, the "8" being hydrated lime; a home-made dust of monohydrated copper sulphate, hydrated lime, and Kayso; a commercial copper-lime dust of the same formula, but without the Kayso; and a Bordeaux dust carrying an electrical charge prepared by the Kil-Tone Company. Five applications of spray were made, and nine of the dusts, the first four of the latter made in the evening and the remainder in the early morning.

Although the protection program was not begun until three or four weeks after scab appeared on the young leaves, yet the dusts held the disease in satisfactory control throughout the season on Alley and Schley, but proved less effective on the Delmas variety. On all varieties the spray mixture produced a higher degree of control than any of the dusts, but tended to accumulate heavily on the leaves during the unusually dry season. Even the spray failed to furnish complete control on any of the varieties owing to the late start in the spray program and to the fact that the first two applications were made with a defective sprayer. The spray mixture surpassed all three dusts in adhesiveness to nuts and foliage. The home-made dust outranked the two commercial dusts, in both adhesiveness and degree of scab prevention. The Kil-Tone dust with the electrical charge exhibited no greater tendency to be retained by the leaves and nuts during the application, and showed even less resistance to washings by rains than the copper-lime dusts. It showed the least amount of control of the three dusts.

All of the untreated Delmas trees lost 100 per cent of their nuts from scab, the sprayed trees 2.9 per cent, and those dusted with the home-mixed dust, the commercial copper-lime dust, and the Kil-Tone dust 21.2 per cent, 54.6 per cent, and 68.7 per cent respectively. The mature nuts from the sprayed Delmas tested 76 per cent marketable regarding the filling qualities, as compared with 91.4 per cent, 83.0 per cent, and 77.8 per cent for nuts from the trees dusted with the home-made dust, the commercial copper-lime dust, and the Kil-Tone dusts respectively. Also, the meats of the sprayed nuts were of poorer quality in respect to plumpness, oil content, color, flavor, etc. The per-

centage of the total number of Delmas nuts that graded marketable was 73.5 per cent for the spray, 71.7 per cent for the home-made dust, 37.5 per cent for the commercial copper-lime dust, and 24.2 per cent for the Kil-Tone dust. The Alley checks lost 9.7 per cent of the nuts from scab as drops, and the Schley checks 3.6 per cent. Only traces were lost from the sprayed or dusted trees of those varieties.

The accumulation of the spray deposit on the leaves under the drouth conditions caused some premature defoliation in all three varieties and undoubtedly accounted for the inferior quality of the sprayed nuts. No injury was noticed from the dust deposits.

Not figuring the price of machines with their depreciation, or the other equipment necessary in connection with spraying pecans, it cost about $6.75 to spray an acre of 18 trees once, $10.20 to dust with the home-made copper-lime dust, $16.35 to dust with the commercial copper-lime dust, and $10.15 to dust with the Kil-Tone dust. With the Delmas variety, the average number of pounds of marketable nuts per tree for the different treatments was as follows: spray 31.2, home-made dust 32, commercial copper-lime dust 12.5, and Kil-Tone dust 10.1. Assuming that the marketable nuts brought the same price, say 40 cents, it would require the following number of pounds of pecans per tree to pay for the different treatments: spray 4.7, and 13, 20, and 13 for the dusts in the same order as listed above.

While these represent results from only one season, and moreover a season of unusual drouth, yet they appear to be quite suggestive in indicating, under the conditions, the comparative fungicidal value of the four materials used, as well as their effect on the function of the tree. Further experiments are being planned to test the effectiveness and practicability of dusting for pecan scab.—Georgia State Board of Entomology Pecan Station, Albany, Ga.

Recent progress in the control of cereal smuts.—W. H. TISDALE.

Considerable progress has been made in recent years in the control of cereal smuts by the Office of Cereal Investigations, U. S. Department of Agriculture, in cooperation with the state agricultural experiment stations of California, Illinois, Kansas, Minnesota, Missouri, New York, Oregon, Wisconsin, and Washington. Seed treatment and varietal resistance are the principal lines of attack. Studies are being made of some of the fundamental factors influencing progress along these lines.

Among the most important developments along the lines of seed treatment was the introduction in 1920 of the copper-carbonate dust treatment for the control of bunt of wheat. This treatment is now being recommended for the control of covered kernel smut of sorghum.

More recently certain organic mercury compounds, including chlorphenol, cresol, and ortho-nitro-phenol derivatives of mercury, have proved effective in the control of some of the cereal smuts. They are especially desirable for the control of barley smuts. They do not cause seed injury, as is true with the standard formaldehyde and copper sulphate treatments.

A single bath hot-water treatment has been devised for the control of loose smut in wheat which is effective and less injurious to the seed than is the modified hot-water treatment. The seed is held, without presoak, in water at 49° C. for 1 hour and 35 minutes, or at 48° C. for 1 hour and 50 minutes.

In the resistance studies, many varieties and selections or strains of the various cereal crops have been subjected to attack by their respective smuts under conditions favorable for heavy infection. The most resistant material from these experiments is used as breeding stock for combining resistance with quality and high yielding capacity.

In some instances resistance has been found associated with these desirable characters. A number of commercially desirable resistant varieties have been discovered or developed through these studies. Among these are Hussar, Martin, Ridit and Sherman wheats which are highly resistant to bunt; Shepherd, Mammoth Red, Trumbull and other varieties of wheat which are resistant to flag smut; Markton, a variety of oat which resists both oat smuts; and Federita, a kernel-smut-resistant sorghum. There are many other varieties and selections or strains of the cereals which are either smut free or practically smut free. Many of them are being used in our breeding program. A variety may be resistant to a certain physiologic form of smut and susceptible to another form of the same species. This complicates our breeding program.

REPORT OF THE SEVENTEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The seventeenth annual meeting of the Society was held at Kansas City December 28–31, 1925, in conjunction with the American Association for the Advancement of Science and affiliated societies. The Hotel Baltimore was the headquarters, and the fact that the meetings were held in the rooms and parlors of this hotel added greatly to the convenience and success of the meeting. It is estimated that about 140 members were in attendance.

Seventy papers were presented at the regular sessions, and ten more were given by members at the joint sssion with Section O on the subject of disease resistance in field crops, thus making a total of 80 papers. Two conferences, one on teaching and the other on extension work in plant pathology, and the usual joint session with Section G, were held. Abstracts of papers were distributed at the meetings and later published in Phytopathology (Phytopath. **16**: 63–88. 1926). A popular account of the meeting, including a summary of facts brought out in some of the more important papers, has appeared in connection with the report of the Permanent Secretary of the American Association for the Advancement of Science (Science 63: 132–133. 1926).

At the joint session with Section G on December 29 four papers were presented, as follows: *Botanical Problems of American Tropical Agriculture,* by W. A. Orton; *The Present Crisis in Plant Physiological Science,* by Burton E. Livingston; *The Nature and Cause of Persistency of Secondary Sexual States with Special Reference to Typha,* by John H. Schaffner; and *Increasing the Life Activities of Plants by Chemical Treatments,* by F. E. Denny.

The subject of teaching plant pathology was discussed at the conference on December 30 by N. J. Giddings, C. W. Edgerton, R. E. Vaughan, and others; and papers by H. H. Haymaker and L. R. Hesler were read.

The Phytopathologists' dinner, which was held at the Hotel Baltimore, December 30, was attended by 174 persons. The program consisted of remarks by the president, C. W. Edgerton; information concerning the coming International Plant Congress, by H. H. Whetzel; the reading of a telegram to Dr. G. R. Lyman, who was unable to attend on account of illness; singing by a quartet and by the assembly, led by Donald Porter; and a two-act play by members of the Society from Ames, Iowa, and Manhattan, Kansas.

Officers and Representatives

The following officers were chosen, the first four being elected by the Society and the others selected by the Council and approved by the Society.

President, I. E. Melhus, Agricultural Experiment Station, Ames, Iowa.

Vice-president, H. B. Humphrey, U. S. Department of Agriculture, Washington, D. C.

Secretary-Treasurer (three years), R. J. Haskell, U. S. Department of Agriculture, Washington, D. C.

Councilor (two years), J. B. S. Norton, Agricultural Experiment Station, College Park, Md.

Associate Editors of Phytopathology (three years), M. W. Gardner, Agricultural Experiment Station, Lafayette, Ind., to succeed W. D. Valleau; N. E. Stevens, U. S. Department of Agriculture, Washington, D. C., to succeed R. D. Rands; H. H. Whetzel, College of Agriculture, Ithaca, N. Y., to succeed W. A. McCubbin; G. W. Keitt, Agricultural Experiment Station, Madison, Wis., to succeed L. W. Durrell.

Business Manager (one year), R. J. Haskell, U. S. Department of Agriculture, Washington, D. C.

Advertising Manager (one year), J. F. Adams, Agricultural Experiment Station, Newark, Del.

Representatives on the Council of the American Association for the Advancement of Science (one year), G. P. Clinton, Agricultural Experiment Station, New Haven, Conn., and Donald Reddick, Agricultural Experiment Station, Ithaca, N. Y.

Member of Editorial Board, American Journal of Botany (three years), G. K. K. Link, University of Chicago, Chicago, Ill., to succeed G. R. Bisby.

Representative on Committee on American Type Culture Collection (one year, 1926, but thereafter for a three year period), C. L. Shear, U. S. Department of Agriculture, Washington, D. C.

Members of Advisory Board (three years), S. M. Zeller, Agricultural Experiment Station, Corvallis, Oregon, to succeed C. W. Hungerford as Commissioner for the West; B. B. Higgins, Agricultural Experiment Station, Experiment, Ga., to succeed L. R. Hesler as Commissioner for the South; F. D. Fromme, Agricultural Experiment Station, Blacksburg, Va., to succeed L. R. Jones as Commissioner at large.

Members of Board of Governors of the Crop Protection Institute (three years), I. E. Melhus, Agricultural Experiment Station, Ames, Iowa, to succeed C. R. Orton; the other members of the Board are M. F. Barrus and N. J. Giddings.

The following temporary committees were appointed by the President to serve throughout the meetings: *Resolutions Committee,* F. D. Fromme and I. E. Melhus; *Auditing Committee,* S. M. Dietz and J. L. Weimer; *Elections Committee,* G. R. Bisby and R. S. Kirby; *Reporting Committee,* W. A. McCubbin and E. L. Nixon.

REPORT OF THE SECRETARY-TREASURER, 1925

During 1925, 46 members were suspended for non-payment of dues, 8 resigned, and 3 died, making a total loss of 57 and reducing the total membership of the Society on December 20, 1925, to 603. Of these 603 members, 109 are life sustaining members and 494 regular members. Sixty-five life memberships were paid up in full December 20, 1925. At the Kansas City meeting 69 candidates were elected, thus making the total membership 672. Of these candidates for membership, 12 came from New York, 7 each from Iowa and Minnesota, 5 from Japan, 4 each from Wisconsin and California, 3 from Canada, 2 each from the District of Columbia, Ohio, Florida, Illinois, Kentucky, and Washington, and the remainder from scattered states.

STATEMENT OF ACCOUNTS FOR 1925, AS OF DECEMBER 19, 1925

Receipts:

Balance from 1924			$3,504.54
Annual dues: 1923	4.00		
1924	12.00		
1925	1,012.02		
1926	1,600.60		2,628.62
Sales received with annual dues			7.00
Excess dues			10.15
Interest: Checking account	59.02		
Time deposit	63.88		122.90
Time deposit transferred to checking account			500.00
Cash for Phytopathologists' dinner tickets			4.00
			$6,777.21

Expenditures:

Amount transferred to Sinking Fund for investment	630.00	
Secretarial work	65.00	
Postage stamps	20.10	
Excess dues refunded	6.00	
Member subscriptions transferred to Phytopathology	3,912.00	
Expenses of Committee on International Botanical Congress.	16.25	
Printing (Preliminary announcements, program, nomination blanks, ballots, bills for dues, Advisory Board stationery)	105.70	
Checks returned by bank	18.00	
Expressage and postage on stationery	2.75	
Complimentary dinner tickets	9.00	
		$4,784.80
Balance		$1,992.41
Outstanding check		16.25
		$2,008.66
Amount of above receipts credited on 1926	1,600.60	
Amount due Phytopathology for European donations	60.98	
Amount due Phytopathology for sales with dues	7.00	
		1,668.58
Actual balance for 1925		$340.08

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1925

PHYTOPATHOLOGY has come through the year with a balance of nearly two hundred dollars in excess of 1924, and in addition has used about thirteen hundred dollars more than last year for expansion. The accompanying statement of accounts shows a bank balance of $2,271.39 and an investment of surplus funds of $1,003.25, which together total $3,274.64.

The present financial status of the journal as compared with that of 1924 is shown graphically on the accompanying chart. Receipts from all sources, with the exception of subscriptions and members' subscriptions, have increased materially over last year. Attention is called especially to returns from advertising and sales of back numbers.

Subscriptions. The number of paid up subscriptions for 1925 on December 20 was 407, of which 246 were foreign and 161 domestic. This is an increase of 29 over 1924. The number of subscribers in good standing at the end of each year for the past five years is shown in table 1. Most of the increase during the past few years has been due to new foreign rather than domestic subscriptions. Russia, Germany and Japan lead in numbers of new subscriptions in 1924 and 1925.

The reduced rates to European subscribers, adopted by the Society at the Washington meeting, have been in force during the year. These prices, based on exchange rates, have resulted in a cost of approximately $140. This has been offset to some extent by the securing of European subscriptions that probably would not otherwise have been obtained. Out of 59 new subscriptions in 1925, 37 were from the European countries concerned, divided as follows:—Russia 14, Germany 9, England 3, Belgium 2,

TABLE 1.—*Subscriptions to Phytopathology*

Year	Number of subscriptions	Increase over previous year
1920	276	—
1921	287	11
1922	320	33
1923	340	20
1924	378	38
1925	407	29

and one each from Austria, Czechoslovakia, Finland, France, Ireland, Italy, Serbia, Spain, and Sweden.

Members' subscriptions. The amount of money received from members' subscriptions this year has been slightly less than last year in spite of an increase in membership. This is largely owing to the use of only $4.00 of each sustaining life membership installment instead of $5.00 as has been done in the past. Also, in 1924, a few life members paid dues in advance.

Advertising. The actual amount of money received from advertising, as shown by the accompanying chart and statement of accounts, was $1,176.32, an increase of $479.03 over last year.

The receipts from the particular advertisements that appeared in the 1925 volume will be about $1140 as compared with $781.50 in that of 1924, which, owing to the activities of the advertising manager, is an increase of $358.50.

Receipts from advertising during the last seven years have been as follows:

1919	$ 221.45
1920	193.83
1921	309.32
1922	519.06
1923	594.57
1924	781.50
1925	1,141.24

Sales. Sales of back numbers more than doubled those of 1924. An unusual number of large orders were received from institutions, especially in foreign countries, for complete sets of the journal. Many requests also came from persons desiring the last few volumes. In all, 80 separate sales of back numbers were made.

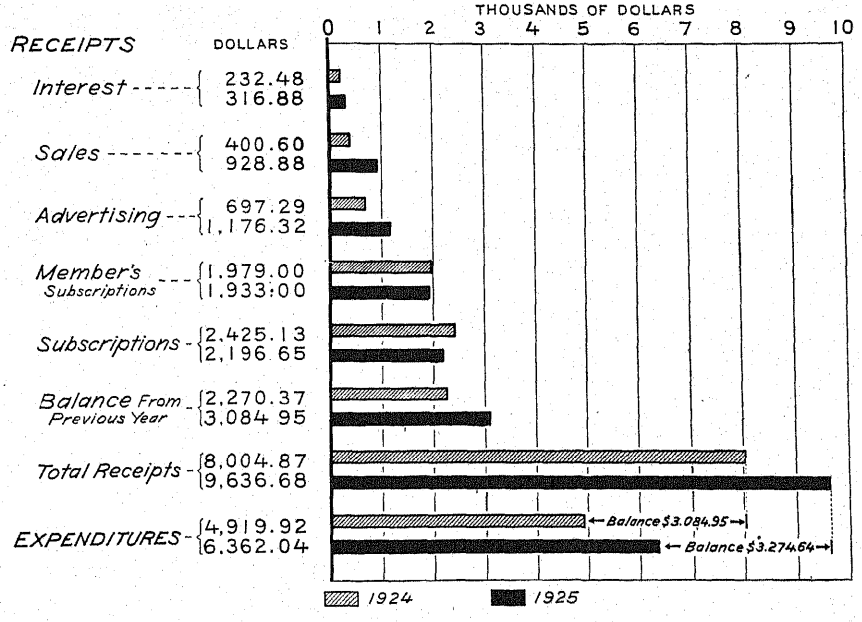
Sinking fund. The sinking fund, resulting from payments of life sustaining members, now amounts to $4,750.00. This is invested in seven mortgage notes ranging in denomination from $500.00 to $1000.00 and bearing 6, 6.5, and 7 per cent interest.

At the Washington meeting the Council authorized the secretary to put $6.00 out of every $10.00 contributed by life sustaining members into the sinking fund, instead of $5.00 as heretofore. This has resulted in an addition to the sinking fund and a diversion from PHYTOPATHOLOGY in 1925 of one dollar for every life sustaining member or $110.00. For 1926 the amount will be about $45.00, as 65 members have now completed their payments.

Expenditures. Owing to the increased size of Volume 15 of PHYTOPATHOLOGY, 732 pages in the first eleven numbers as compared with 536 pages in the corresponding

numbers of last year, the cost of manufacture was considerably greater than last year. It cost $5,102.62 to prepare numbers 1 to 11 of Volume 15 and $3,801.20 for the same numbers of Volume 14, a difference of $1,301.42. The increased cost is entirely due to the greater number of pages and cuts, as printers rates and costs of paper have remained the same. Volume 15 is by far the largest ever issued.

PHYTOPATHOLOGY RECEIPTS AND EXPENDITURES 1924 AND 1925



The increased postage rates on second class matter which went into effect January 1 of last year have resulted in an increased cost over last year of about $36.00 for mailing back numbers from Washington and College Park, Maryland, where back volumes are stored. The increased rates did not affect the publisher at Lancaster, but owing to the greater weight of this year's volume, the cost of mailing the first eleven numbers was $223.25, or $64.14 in excess of 1924.

Another item of expense which appears for the first time this year is that of insurance on back volumes of Phytopathology that are stored at College Park, Maryland. Although located in a building that is supposed to be fireproof, they are stored in a room with bulletins and other inflammable matter, and so it seemed advisable to insure them against fire. Accordingly a policy, costing $65.00 and extending over a three year period, was purchased.

STATEMENT OF ACCOUNTS FOR 1926, AS OF DECEMBER 19, 1925

Receipts:

Balance from 1924	$1,105.95
Subscriptions ($706.99 for 1926)	2,196.65
Sales	928.88

Advertising, 1924	174.01	
1925	962.98	
1926	39.33	1,176.32
Interest on invested sinking fund		316.88
Balance due sinking fund		630.00
Principal of Kaulbeck mortgage note paid		1,000.00
Member subscriptions from Society for 1924		1,979.00
Member subscriptions from Society for 1925		1,933.00
		$11,266.68

Expenditures:

Manufacturing Phytopathology:		
Vol. XIV. No. 12	321.91	
Vol. XIV, Index	148.65	
	470.56	
Vol. XV, No. 1	362.58	
Vol. XV, No. 2	383.79	
Vol. XV, No. 3	369.76	
Vol. XV, No. 4	332.78	
Vol. XV, No. 5	457.28	
Vol. XV, No. 6	228.99	
Vol. XV, No. 7	485.61	
Vol. XV, No. 8	391.49	
Vol. XV, No. 9	380.64	
Vol. XV, No. 10	429.22	
Vol. XV, No. 11	534.85	
Engravings for Vol. XV	745.63	
	5,102.62	
		$5,573.18
Printing Phytopathological Abstracts		14.51
Expenses of Editor in Chief (including letterheads, postage, etc)		40.43
Expenses of Advertising Manager (including envelopes, advertising sheets, printing stationery)		80.46
Second class postage (mailing back vols. from College Park)		33.98
Secretarial work		260.00
Postage stamps		23.00
Sinking fund invested with accrued interest		1,760.79
Printing letterheads, billheads for Business Mgr.		34.71
Purchase of stamped envelopes		56.85
Insurance on stock of back volumes		65.20
Miscellaneous expenses, account books, telegrams, graphs, paper and labor in tying back volumes		32.43
Duplicate subscriptions refunded		16.00
Excess advertising refunded		.50
Investment of surplus funds with accrued interest		1,003.25
		$8,995.29

	$8,995.29
	$2,271.39
Outstanding check	15.00
Balance	$2,286.39

Assets:

Cash in bank (balance as shown in statement of accounts)	$2,286.39
Sinking fund (invested)	4,750.00
First mortgage (surplus)	1,000.00
Amount due from sales made	59.00
Amount due from advertising	160.80
Amount due from subscriptions	317.75
Member subscriptions to date	1,081.00
Cash and checks on hand to be deposited	88.96
Sales received with member's dues (now in Society account)	7.00
Amount due Phytopathology for European subscriptions	60.98
Total assets	$9,811.88

Liabilities:

None.

REPORT OF THE EDITOR-IN-CHIEF OF PHYTOPATHOLOGY

Volume 15 of Phytopathology contains 810 pages, comprising 86 articles, 23 notes, 7 reports, 3 book reviews, 40 plates, and 140 text figures. About 150 manuscripts, including notes, were submitted. Of these, 35 were returned to the authors for revision, and 6 were rejected or withdrawn under pressure. It is the largest volume ever issued. While it is gratifying to have published so much material, the Editorial Board assumes no particular credit, except that it took advantage of the congestion of several other journals and invited pathologists to submit their papers to Phytopathology, with the promise of relatively prompt publication. The fact that there were sufficient funds to publish so much material is due to the energy, efficiency, and effective business sagacity of the Business Manager and the Advertising Manager. The contribution of $350 by the Graduate School of the University of Minnesota for editorial assistance also enabled the editors to handle more manuscripts than they otherwise could have done. The editor wishes also to acknowledge the accommodating helpfulness of A. E. Urban, Manager of the Science Press Printing Company, in the onerous task of issuing the journal.

Unfortunately, not all of the numbers were issued on time, owing partly to the readjustments necessitated by the change in personnel of the Editorial Board, partly to the delays caused by sending poorly prepared manuscripts back and forth for revision, and partly to perfectly natural causes.

Most papers were published in order of receipt, although the exigencies of "make up" often makes it difficult to fit papers into the number for which they are scheduled. And it should be perfectly clear that poorly written manuscripts which require extensive editing and revision can not be sent to the printer as promptly as those which are ready for publication when received. Much time is consumed in editing poorly-written manuscripts and sending them back and forth between author and editor, a process which often must be repeated several times. On the other hand, when few or no changes are necessary, manuscripts can be marked for the printer and sent merrily on their way without delay.

During the past year the Editorial Board has not discriminated against papers on account of length. It seemed far better to publish good papers, even though long, than to clutter up the journal with many short but mediocre contributions. Not that there is necessarily any merit in length; some people apparently try to make a paper long merely in order to have a few more published pages to their credit or to appear erudite. This is deplorable but true. The shorter the paper, provided the brevity is compatible with completeness and clearness, the better the impression it makes. However, short papers should not degenerate into mere news items, plant disease survey notes, or notices that a field of investigation is preempted.

A discussion regarding the quality of papers acceptable for Phytopathology raises a delicate question. There seems to be little unanimity of opinion as to what constitutes a valuable paper. The Editor took a ballot on the quality of papers appearing in several numbers of Phytopathology, and, if the results had been plotted, a corkscrew curve would have resulted. What seems superfluous and piffling to some may seem important and valuable to others: a paper may be fertile with information and ideas for some pathologists but completely barren for others.

Borderline papers constitute a real problem, often resulting in considerably increased nocturnal wakefulness on the part of members of the Editorial Board. To do justice to an author and at the same time maintain a high standard for the journal is sometimes a difficult equilibristic feat. When the Editor was in doubt regarding the acceptability of papers submitted, he asked the opinion of as many different individuals as possible.

No attempt is made to compete with the Atlantic Monthly in excellence of style. Style, after all, is a somewhat elusive thing, but it does not require a stylist to observe that some papers have no style at all. Readers have the right to expect that scientific results will be presented clearly, concisely, and consecutively. It should be possible for a reader to get the writer's meaning without doing literary detective work. Ambiguity, prolixity, redundancy, and deadening monotony of expression completely spoil many manuscripts. Certainly the first three have no place whatever in scientific papers. Even an amateur editor can detect such glaring faults; in fact they often overwhelm him with their obviousness. Some authors have intimated that insistence on correctness of expression and punctuation are carried to extremes of picayunishness. Some have insisted that the reader can unravel a sentence about half a page long, containing six or seven subjects, fifteen or twenty modifiers and a score of verbs, half of which are not in agreement with the subject. Maybe so. The reader probably could solve a cross-word puzzle too, but scientific journals are not designed to furnish that sort of mental test. Papers should be written clearly enough to enable a reader to get the meaning quickly and as painlessly as possible.

The readers have a right to expect that every paper shall contain an introduction to the subject, a clear-cut statement of the objects of the investigation, a complete but concise statement of the results, a discussion of the significance of the results, and a brief but comprehensive summary.

There should be some references to literature cited. There seems to be an increasing tendency, as pointed out by the previous Editor-in-Chief, for many pathologists to disregard previously published results. With the tremendously increased volume of literature, the formulation of a policy with respect to literature citations is difficult. But it does seem that reference should be made at least to some previous papers which have summarized the contributions made up to the time they were published.

The quality of papers submitted for publication in Phytopathology should be improved. This is true both of substance and form. A paper should contain a con-

tribution to knowledge or thought, and the material should be well presented. Unfortunately, scientific manuscripts usually are written by professional scientists but amateur writers. And they show it. In many of the papers the most elementary rules of English composition are violated; and others are full of obvious typographical errors which the authors should have corrected before the manuscripts were submitted. There is a deplorable tendency on the part of authors to exceed all speed limits in rushing their papers into print. This is a perfectly natural desire with which the Editor sympathizes, but it is not fair either to the paper or the author—to say nothing about the Editor or the readers. No one has a right to throw his results together in the most haphazard way and expect an editor to polish his paper for him. Some authors try to make their major revision on the galley proof, or even on the page proof. The time to write a scientific paper is before it is published and not afterwards.

Every author, no matter how experienced he may be, should submit his manuscript to some colleague before submitting it for publication. There would be fewer retractions and explanations if this were done. About 90 per cent of the authors should enlist the aid of a professor of English or an experiment station bulletin editor. If they did, their papers would be published more promptly and would be more favorably received by the long-suffering reading public.

It should be remembered, also, that the members of the Editorial Board of Phytopathology are not professional editors; consequently they also make mistakes. The guide posts on the road to a better Phytopathology say, better papers and fewer mistakes, all along the line.

REPORT OF THE ADVERTISING MANAGER

The total number of advertisements for the twelve issues during 1925 was 118, or an increase of 49 per cent over 1924. This total of advertisements was distributed as follows: 46 full page; 38 one-half page; 16 one-fourth page; and 10 one-eighth page, making a total of sixty-nine and one-fourth pages of advertising or seventeen and one-fourth pages over 1924, representing a 33 per cent increase.

It was necessary to carry on an extensive amount of correspondence in order to secure this small increase. In order to lighten the correspondence and broaden the interest of PHYTOPATHOLOGY as an advertising medium, a campaign with the slogan "Add an Ad in Phytopathology" was launched. Attractive stickers with this slogan were printed and distributed to departments for use by members on correspondence, requisitions, etc., to concerns which should be attracted to our publication for advertising. Through the cooperation of two members, this campaign resulted in securing advertisements from only two concerns, which was sufficient to pay the relatively small expense of the campaign. It has been very difficult to secure recognition from companies manufacturing spray, laboratory, and microscopic equipment. It is urgently requested that all members, if at any time in contact with various commercial representatives, take the opportunity of presenting the merits of PHYTOPATHOLOGY as a practical and efficient publication in which to advertise. A reasonable advertising increase for 1926 can only be anticipated by personal cooperation of members.

REPORT OF THE ADVISORY BOARD

The Advisory Board made the following report through its Chairman, M. F. Barrus.

The personnel of the Board of 1925 is as follows: F. C. Meier, Secretary, representing the U. S. Department of Agriculture; N. J. Giddings, representing the Northeast; L. R. Hesler, representing the South; M. W. Gardner, representing the Midwest; C. W. Hungerford, representing the West; J. E. Howitt, representing Canada;

and L. R. Jones and M. F. Barrus, Chairman, members-at-large. The Council has appointed the following to the Advisory Board for 1926: S. M. Zeller to succeed C. W. Hungerford, B. B. Higgins to succeed L. R. Hesler, and F. D. Fromme to succeed L. R. Jones. At a meeting of the Advisory Board, N. J. Giddings was elected chairman for 1926.

Summer Meetings. Two summer meetings of the Society were held in 1925, one at Corvallis, Oregon, June 22–24, in conjunction with the annual meeting of the Pacific Division, and a cereal conference in Iowa, Minnesota, and North Dakota, held July 8 to 14. Brief reports of these meetings have been published in PHYTOPATHOLOGY.

The Advisory Board has decided to hold the summer meeting for 1926 at Ithaca, August 21 and 22, in connection with the meeting of the International Congress of Plant Sciences.

Tropical Research Foundation. The following report has been received from W. A. Orton, Director and General Manager of the Foundation:

"The leading project, investigations of sugar cane production problems in Cuba for the Cuba Sugar Club, an association of 57 of the leading sugar mills in that country, has now been in progress for one year. A well equipped experiment station building has been erected at Central Baragua, Camagüey Province, near the center of the Island, and experimental fields laid out and planted. A branch station for varietal studies has been established at Herradura, Pinar del Rio, and field experiments on mosaic have been located at two other points, Jatibonico and Jobabo. A general survey has been made of the cane areas of Cuba, and a reconnaissance soil survey cooperative with the Bureau of Soils is nearly completed. This work is in charge of Mr. D. L. Van Dine, Field Director, with the following staff: James A. Faris and Marion N. Walker, Pathologists; C. F. Stahl, Entomologist; R. V. Allison, Chemist and Soil Biologist; S. Lomanitz, Chemist; F. S. Earle, Sugar Cane Technologist (half time); A. V. Svitzer and Luis Scaramuzza, Field Assistants.

"Our energies are concentrated in the main on four important problems; mosaic, root disease, cane borer, and cane varieties.

"The second project, forestry investigations in Cuba, was begun on November 1, 1925. This involves a reconnaissance survey of eastern Cuba, which is supported by the United Fruit Company and the Cuba Company. The scientific staff includes the following: Dr. H. N. Whitford, Mr. Thomas H. Gill, Mr. William Crosby, and Mr. Donald Matthews, the first two for a temporary period.

"The Foundation is about to send an expedition to the west coast of Peru to advise the *Sociedad Agraria* relative to experiment station problems and organization for sugar cane and cotton."

National Research Council. The chairman of the Board attended a meeting of the Division of Biology and Agriculture held at Washington during April. The following projects reported at that time and later have some relation to plant pathology:

"In order to hold the representation on the Division to an effective working body, to keep expenses within reasonable limits, and to maintain representation of the national research societies," a plan has been proposed whereby the present membership may be reduced by means of group representation. By this plan, which was later approved by the executive committee of the Division, The American Phytopathological Society is grouped with The Society of American Bacteriologists. These two societies will select one representative on the Division. This plan is to be voted on at the next annual meeting of the Division.

The Committee on Phytopathology in the Tropics has concerned itself primarily with the promotion of a plan for a graduate school of tropical agriculture, with an

associated botanical garden and research biological station, to be located in southern Florida. Dr. W. A. Orton has recommended that, as it may be found desirable to broaden the scope of the proposed station, a new committee be formed to take the place of the old one, and this suggestion was approved by the Division. The new body is called the Committee on Subtropical Biological Institution. The personnel of this committee is W. A. Orton, Chairman, M. M. Metcalf, and R. A. Harper.

The Committee on Research Publications is a joint committee with the Union of American Biological Societies with Dr. C. E. McClung as chairman, appointed to ascertain the needs of the various research journals in biology.

The Joint Publication Committee is composed of four members appointed by the Union of American Biological Societies, four by the Division of Biology and Agriculture, one by the American Association for the Advancement of Science, one member-at-large, and a representative of the English biologists. Meetings have been held for discussion and action on phases of the Biological Abstracts Journal.

Official endorsement of the Biological Abstracts Journal has been made by the British Association for the Advancement of Science and the Australian Research Council, and unofficial assurance of cooperation by the Japanese Research Council. Dr. J. R. Schramm as editor-in-chief is engaged in getting the journal under way and abstracting will be begun with the 1926 literature, with publication of abstracts later in the year.

The Arthur Rust Project was reported as follows by Dr. C. R. Orton:

"Dr. J. C. Arthur and Dr. F. D. Kern during July and August visited the leading rust specialists of Europe, including Dietel, Sydow, Eriksson, Jorstad, and Butler. Expenses for Dr. Arthur were paid by himself and of Dr. Kern by special arrangement of Pennsylvania State College. The purpose of the trip was (1) to discuss various theoretical problems connected with taxonomy and morphology, (2) to discuss nomenclature especially as regards Dr. Arthur's terminology *vs* the old terminology. The results of this visit are (1) general agreement on the first point (2) division of agreement on the second point, a majority of the Europeans thinking the new terms unnecessary.

"The persons selected to write the rust book are Drs. Arthur, Kern, Fromme, Bisby, Mains, Jackson and Orton. The book has been completed except for a chapter on control of rusts, a short chapter on teretological features of rusts, and a chapter on classification."

Information Service in Plant Pathology. Last year the Advisory Board recommended that a plan be worked out for supplying the layman with information and news about plant pathology. The Council approved this suggestion and appointed W. A. McCubbin to consider the possibilities of developing such a service and, if it seemed feasible, to prepare a plan. Dr. McCubbin submitted such a plan to the members of the Advisory Board, who examined it critically and offered certain suggestions. Some of these are included in a revised plan presented to the Society at the Kansas City meetings and approved by it. The Council, upon the recommendation of the Advisory Board, has appointed W. A. McCubbin, F. C. Meier, and G. R. Lyman to a Committee on Information Service in Plant Pathology responsible to the Council, and has appointed W. A. McCubbin as publicity editor, with recommendation that the service be inaugurated.

Investigations on Plant Diseases and Fungicides Conducted by the Crop Protection Institute. The cereal seed treatment project was discontinued at the end of last year. The results of the work have been prepared for publication in PHYTOPATHOLOGY.

Furfural investigations were terminated on March 1, 1925. A paper, setting forth the studies on furfural, has been prepared for publication in PHYTOPATHOLOGY.

Scalecide investigations are supervised by a committee consisting of H. W. Thurston E. L. Nixon, P. J. Parrott, and H. W. Anderson. The main part of the investigation is being conducted by Mr. John Miller with headquarters at Pennsylvania State College. Studies are also being made in Massachusetts, and Illinois in cooperation with the Agricultural Experiment Stations of these respective states. The B. G. Pratt Company has provided $13,500 to continue the investigation for a period of three years. Prof. Thurston reports that the investigations thus far have indicated scalecide to have but little effect in stimulating or inhibiting the growth of apple tree. No definite effect on fire blight cankers on body and root has yet been observed. Studies are being made on the penetration of scalecide into the tissues of the apple tree in the hope of throwing some light upon oil injury. All three lines of investigation will be continued another year.

The crown gall investigations project is supervised by a committee consisting of I. E. Melhus, G. W. Keitt, and M. F. Barrus. The investigators are A. J. Riker and J. H. Muncie. Headquarters for the work are at Wisconsin Agricultural Experiment Station and Iowa Agricultural Experiment Station. Funds for the investigation amounting to $6000 a year have been furnished by the American Association of Nurserymen, by individual nurserymen, and by the Universities of Iowa and Wisconsin. The project has run for nearly two years and will terminate on June 30, 1926, unless additional aid is forthcoming. Some of the results of this investigation have already been published. Papers were presented at the Kansas City Meeting by Dr. Riker and Dr. Muncie. These and others will be published later.

The copper investigations project is supported by the Nichols Copper Company, the Baalbach Metals Corporation, the Goldsmith Brothers Smelting and Refining Company, and the Boyce-Thompson Institute for Plant Research, Inc. by Dr. Frank Wilcoxon under the supervision of a committee consisting of Drs. William Crocker, N. J. Giddings and R. W. Thatcher. The following statement of progress has been presented by Dr. Crocker.

"This investigation was commenced in May, 1925, and had for its immediate objective the preparation of a reversible colloidal copper fungicide which could be sold in concentrated form, and could be prepared for use by merely stirring into water. It was restricted to such compounds as would meet the requirements of a satisfactory fungicide as regards toxicity, cheapness, adhesiveness, harmlessness to foliage, etc. The work involved laboratory tests for toxicity, and adhesiveness, as well as the preparation and examination of a large number of copper-containing substances. Of these, two have been found which seem to warrant more extensive tests in the field, and it is hoped to carry out such tests next year."

The seed-borne parasites and seed disinfection project is supported by funds, amounting to $3,500 a year, appropriated by The Bayer Company and is conducted in cooperation with The Boyce-Thompson Institute for Plant Research at that Institute. The investigator, C. R. Orton, devotes half time to the project. The committee consists of William Crocker, who supervises the project, M. T. Munn, and W. L. Burlison.

An application for the incorporation of the Crop Protection Institute under the laws of the District of Columbia has been made and a revised Constitution and By-laws of the Institute to accord with the requirements of the law have been submitted to the members for a letter ballot.

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

Auditing Committee. The Auditing Committee, S. M. Dietz, and J. L. Weimer, reported as follows:

The books of the Phytopathological Society were audited and checked with the resume presented before the Society on December 30, 1925.

The books of PHYTOPATHOLOGY as presented before the Society on December 30, 1925, were audited and found correct.

Representative on Committee on Plans for International Congress of Plant Sciences. H. H. Whetzel outlined the plans for the forthcoming Congress at the dinner on the evening of December 30. A summary of his remarks follows:

"The International Congress of Plant Sciences meets at Ithaca, N. Y., August 16–23, 1926. There will be some thirteen or more sections representing all branches of plant science, each with an executive secretary. Dr. D. Reddick is the Secretary of the Phytopathological Society and the program for this society is in his charge. The program will be made up of invitation papers, but ample opportunity for round table and general discussion will be provided.

"Field and collecting trips are being planned, at least one trip to nearby sections in the State where diseases of crops, especially of truck and field crops, may be studied. These will be arranged for by the officers in charge of the summer meeting of the Society.

"Cornell University will provide rooms and cafeteria service at a very reasonable charge and can handle all comers. A special effort should be made to make this the greatest international gathering of plant pathologists to date. Every American plant pathologist should plan to be there."

Representative on American Type Culture Collection Committee. Miss Margaret B. Church, in the absence of the Society's representative, C. L. Shear, presented a report embodying the following information:

"Maintenance of the fungi in the American Type Culture Collection is supervised by Drs. Charles Thom and Margaret B. Church of the U. S. Department of Agriculture, Bureau of Chemistry, Washington, D. C. The Microbiological Laboratory in this bureau has been maintaining successfully saprophytic molds in culture for numbers of years. The executive committee of the collection assigned to the project about $400 per year to be used by an assistant. This provides about ten hours per week service. When the temporary mimeographed list of the collection was issued in September 1925 the number of fungi included was about seventy. Other cultures of fungi have been and will be added as time and facilities permit. Cultures included are as free from contamination as can be ascertained and are identified by competent persons. Cultures are transferred in Washington and mailed to Chicago for distribution. New transfers are periodically sent to Chicago to replace the older ones. Current record books, a letter file and a card index are developed for the collection. The card index indicates the name of the fungus, the source of the particular culture, the date of its isolation, and the original citation. This collection of living cultures of fungi is of active interest to students, investigators, and those dealing with industrial and agricultural problems."

Resolutions Committee. The chairman of this committee, F. D. Fromme, presented the following resolutions, which were unanimously adopted:

1. *Resolved:* That the American Phytopathological Society expresses its appreciation of the progress being made by the Committee on American Type Culture Collections in providing supplies of pure cultures of bacteria and fungi, and urges that the collection of fungi, especially plant parasites, be increased as rapidly as possible.

2. WHEREAS: Plant pathologists have found the recent "Author and Subject Index to the Publications on Plant Pathology of the U. S. Department of Agriculture" very useful, and whereas a similar index of publications of State Experiment Stations would be equally helpful, therefore be it

Resolved: That the American Phytopathological Society assembled at Kansas City respectfully requests the Bureau of Plant Industry Library to consider the preparation of an author and subject index to the experiment station literature in the same manner as has just been done for the U. S. Department of Agriculture publications, and that copies of this resolution be sent to the Chief and to the Librarian of the Bureau of Plant Industry.

3. *Resolved:* That the editor and the editorial board of PHYTOPATHOLOGY are especially commended for the vigorous and heroic policy of improvement which they have inaugurated and followed during the past year; our support is assured for the application and further extension of this policy during the coming year. It is recognized that the excellence of the journal is due in no small measure to the activities and good judgment of the Business Manager and the Advertising Manager, and we further record our sincere appreciation of their work.

4. *Resolved:* That we record a most favorable reaction to the hotel type of convention and express a desire that arrangements for similar hotel meetings be made in the future when facilities permit.

5. *Resolved:* That we hereby express our appreciation of the excellent facilities for the business of our meetings and for entertainmnts that have been provided at Kansas City. This expression is especially tendered to the general officers of the American Association for the Advancement of Science; to the local committee for the Kansas City meeting; to the officers of the American Phytopathological Society and to its program committee; and to the management of the Hotel Baltimore.

6. *Resolved:* To the authors, producers and entertainers who are responsible for our dinner program, and to the founders of the Epsilon Phi Tau fraternity we express our thanks for a most enjoyable evening.

ACTION OF THE COUNCIL

In addition to the appointments mentioned in the first part of this report, the Council reported the following actions which were approved by the Society.

It was recommended that there be initiated the publication of a series of separate papers, independent of Phytopathology, consisting at the outset of translations of important classical articles. This series might be appropriately termed "Phytopathological Classics." These papers are to be sold at a profit sufficient to provide for the continuation of the series. The work of obtaining and preparing them is to be entrusted to an editorial committee of three consisting of H. H. Whetzel, Chairman, E. C. Stakman, and F. D. Fromme.

The Council recommended the appropriation of $300 from the treasury of the Society for the use of the International Congress of Plant Sciences, one-hundred dollars to be for the use of our representatives on the committee, and the sectional secretary, and the remaining $200 to be applied to the general fund.

Attention of members was called to the Pan-Pacific Science Congress which will be held at Tokyo, Japan. October 27 to November 9, 1926. Any members who think they will be able to attend are asked to communicate with the president or secretary in order that they may be properly appointed as delegates.

The Council adopted rules of procedure, proposed by the committee on Necrology, to be followed in connection with receiving the report of that committee and in obtaining and preparing biographies of deceased members. These rules are as follows:

1. The Committee recommends that the time of receiving the annual report of the Committee on Necrology be carefully chosen by the President or presiding officer with respect to full attendance and the avoidance of too great disharmony with what precedes and follows on the program.

2. The Committee recommends that the Society inaugurate the custom of standing while the annual report of the Committee on Necrology is received, or if this recommendation fails of approval, that the Council consider and inaugurate some other method of giving an appropriate degree of solemnity and distinction to this occasion.

3. The Committee recommends that a brief, but full page notice *in memoriam* be annually printed in the number of PHYTOPATHOLOGY in which the annual meeting is reported, following the custom inaugurated in the number of PHYTOPATHOLOGY, for May, 1925.

4. The Committee recommends that a suitable biography of every deceased member shall be published in PHYTOPATHOLOGY; that this shall, at the discretion of the editor-in-chief, be accompanied by a photograph and by a complete, carefully prepared bibliography, or a citation of a complete bibliography published in some other journal. It is further recommended that the Committee on Necrology shall be responsible for seeing that such a biography is suitably prepared and submitted for publication with reasonable promptness.

5. The Committee recommends that ten copies of the number of PHYTOPATHOLOGY in which any given biography appears, or ten reprints of the biography, shall be sent to the family of the deceased at the expense of the Society.

Committee on Necrology—Haven Metcalf, G. P. Clinton, and L. R. Jones.

MISCELLANEOUS BUSINESS

The reports of the various officers and committees as given in the preceding pages of this report were adopted. The Constitutional amendments as printed in PHYTOPATHOLOGY (Phytopath. 15: 619–620, Oct. 1925) were adopted. The secretary's report of the last annual meeting as presented in PHYTOPATHOLOGY (Phytopath. 15: 304–319, May, 1925) was accepted.

It was voted to hold the next annual meeting of the Society at Philadelphia in conjunction with the American Association.

In connection with the discussion for improving the quality of articles published in PHYTOPATHOLOGY, it was voted as the sentiment of the Society that the editor and management be authorized to announce that no papers would be accepted for publication in PHYTOPATHOLOGY that do not have the signature or approval of some member of the Society, or some botanist of recognized standing, this announcement and procedure to be at the option of the editor.

A motion to discontinue the printing of abstracts of papers that will be presented at the next annual meeting was lost, as was also the motion to print only abstracts of those papers that are actually presented at the meeting. In connection with a discussion of the program, it was voted, on motion by L. R. Jones, that it was the sentiment of the meeting that as far as possible the program committee be authorized to take steps to dignify, systematize and simplify the programs. It was also urged strongly that members should not present abstracts and titles unless they fully intend to be present at the meeting to present their papers.

It was suggested that, since simultaneous sessions appear to be necessary and desirable, chairmen of these sessions be appointed at an early date in order that they may take part in arranging the programs.

Notice was given of the annual meeting of the Southern Division, which will be held this year at the Biltmore Hotel, Atlanta, February 3, 4 and 5.

R. J. HASKELL, *Secretary.*

IN MEMORIAM

ELLSWORTH BETHEL, 1863–1925

"Professor" Bethel, as he was universally called, graduated from Grant Memorial University, Athens, Tennessee, in 1886. The University of Denver conferred on him the honorary degree of Master of Arts in 1905. From 1888 to 1917 he taught in the public high schools of Denver, Colorado. From 1917 until his sudden death he was a pathologist in the Office of Forest Pathology with headquarters at Denver. Self-taught in natural history, he began field work as an amateur, and achieved world-wide reputation as a collector. While his specialty was the Uredineae, he covered the field of botany and invertebrate zoology. His name is perpetuated in the Hymenopterous genus *Betheliella,* and the specific name *betheli,* applied to at least twenty species—rusts, flowering plants, fossil fungi, insects, mollusks, and even a variety of *Peripatus.* We remember him for his generosity to correspondents and to visiting scientists, to whom he delighted to unfold the scientific resources of Colorado.

FRANK JOSEPH PIEMEISEL, 1891–1925

Frank Joseph Piemeisel received the degree of Bachelor of Science in Agriculture from the University of Minnesota in 1914, and the degree of Master of Science in 1915. He was then employed by the Office of Cereal Investigations, and had made arrangements to pursue graduate work for the doctorate, when, on September 20, 1917, he was drafted into the army. During his service overseas with the Meteorological Section of the Signal Corps he contracted tuberculosis, and after 13 months returned to the United States, utterly incapacitated. In 1925, at the age of 34, he died of tuberculosis at the Veterans' Bureau Hospital in St. Cloud, Minnesota.

A serious minded student in whom the desire and joy of study was inborn, Frank Piemeisel demonstrated his outstanding ability in research while in the Office of Cereal Investigations, an ability which predicted leadership in the field which he had chosen for his life work.

IN MEMORIAM

JOSEPH ROSENBAUM, 1887–1925

Born in Russia, descendant of a long line of Jewish Rabbis, Rosenbaum came to America at the age of nine. He was graduated in 1911 and received the degree of Doctor of Philosophy in 1914 from Cornell University. In 1915 he was instructor in Plant Physiology in Cornell: from 1915 to 1920 with the Bureau of Plant Industry, engaged mainly in the study of potato and tomato diseases. From 1920 until his untimely death he was in charge of the department of Biology at the Milford Preparatory School, Milford, Connecticut. For one whose years in productive research were so few, he made a notable number of contributions to science.

MARK ALFRED CARLETON, 1866–1925

Mark Alfred Carleton received the degree of Bachelor of Science from the Kansas Agricultural College in 1887, and the degree of Master of Science in 1893. He held successively the chairs of Natural History at Garfield University and at Wichita University and was for a time Assistant Botanist at the Kansas Experiment Station. In 1894 he became a pathologist in the U. S. Department of Agriculture. From 1901 to 1918 he was Cerealist in Charge of the Office of Cereal Investigations, except for a brief period in 1913–14 when he was furloughed to take charge of the chestnut blight control campaign of the State of Pennsylvania. Taking up commercial work in 1918, he was successively with the U. S. Grain Corporation, the United Fruit Company, and at the time of his death with the Laboratorio de Plagas Algodoneras at Piura, Peru.

Carleton was best known as an agronomist, in which line of work, by the introduction and establishment of durum wheat, Swedish Select oat, Khakof wheat and other grains, he made monumental contributions to the prosperity of the United States. But his early work and his last was in plant pathology; and his achievements in agronomy were the outgrowth of his studies on the Uredineae. "He established the physiological relationships of nearly all the cereal rusts of this country and demonstrated the distinctness of the different forms of the same species of these rusts adapted to the same hosts, traced the yearly cycle of the orange leaf-rust of wheat, and showed that durum wheat, emmers, einkorns, and some other wheats are more or less resistant to rust." His position in science and in agriculture is secure, resting solely upon his own achievements.

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STUDIES OF THE EPIDEMIOLOGY AND CONTROL OF FIREBLIGHT OF APPLE[1]

A. N. Brooks

Fireblight, caused by *Bacillus amylovorus* (Burr.) Trev., is one of the most destructive diseases of pomaceous fruits. It has been recognized in America for more than 125 years and has been the subject of much investigation for a half century. During the period from 1877 to 1882 Burrill (5, 6, 7) discovered the bacterial nature of the disease and named the causal organism. Arthur (3) isolated the organism and by inoculation tests further established its causal relationship to the disease. He also recommended as a control measure the cutting out of blighted parts of the host plant. Waite (40, 41, 42) showed that the blight organism is spread from blossom to blossom by bees and that the disease might be controlled by means of an adequate program of excision of diseased parts where the work covered a sufficiently large area. From time to time experiments and observations have been made upon the modes of dissemination of blight. Stewart (36, 37, 38), Burrill (4), Jones (24, 25), and others demonstrated the spread of twig blight by means of sucking and boring insects. Stevens (34) suggested the possibility of the spread of the disease through wind-borne inoculum, and Gossard and Walton (17) showed that rain is a factor, at least locally, in the dissemination of blight. During the past 15 years much work has been done in an attempt to improve methods of blight control. Reimer (31) and Day (10, 11) have contributed toward the betterment of methods of excising blighted parts. Reimer (30) has made sub-

[1] Approved for publication by the director of the Wisconsin Agricultural Experiment Station.

Grateful acknowledgments are made:

To Dr. G. W. Keitt for his kind supervision of the experimental work and suggestions and criticisms in the preparation of the manuscript.

To Dr. A. J. Riker for suggestions and aid in initiating the work.

To the managers and officers of the Weston Orchards Company of Menomonie and the Kickapoo Development Company of Gays Mills for their cordial cooperation.

stantial progress in combating pear blight by the use of disease resistant stock.

In Wisconsin, epidemic outbreaks of fireblight of apple are common and destructive. Numerous attempts on the part of apple growers of this State to control the disease by the excision method have met with indifferent success. Whether this is to be attributed to improper execution or to inadequacies of the method under Wisconsin conditions has been subject to question. In view of these facts further investigations under local conditions appeared to be desirable. This work was directed along two major lines: (a) a detailed study of the seasonal development of the fireblight organism and of the disease in relation to host development and to certain environmental factors, and (b) tests of certain control measures. The work was carried on at Madison, Wisconsin, during the winters 1923–24 and 1924–25 and in field laboratories located in orchards near Menomonie and Gays Mills during the growing seasons of 1924 and 1925.

STUDIES OF THE OVERWINTERING OF BACILLUS AMYLOVORUS

A more detailed knowledge of the mode and abundance of overwintering of the blight organism under Wisconsin conditions seemed fundamental to an adequate understanding of the epidemiology and control of the disease. Consequently the following studies were initiated.

Overwintering on wild hosts

A study of wild hosts of *B. amylovorus* in the vicinity of the experimental orchards, chiefly *Pyrus coronaria* L. and *Crataegus* spp., did not reveal any cases of overwintering of the bacteria. In view of this fact and of the abundance of overwintering in the commercial orchards, as reported below, wild hosts are not considered to have played any significant part in overwintering the organism under the conditions of these studies. It should be noted, however, that wild hosts of *B. amylovorus* are comparatively sparse in their occurrence in Wisconsin.

Variety of apple in relation to overwintering

One of the first problems of overwintering was to learn the relative importance of various commercial varieties in carrying over the organism from season to season. With this in mind, observations were made in the early spring of 1924 at Weston Orchards and at the orchard of the State Horticultural Society near Menomonie, and in 1925 at Kickapoo Orchards near Gays Mills, Wisconsin.

The chief criterion of overwintering was the production of exudate on cankers and twigs which had been blighted the previous year, although the

extension of blighted areas was also used to a less extent. In the latter case, where there was no production of exudate, the identification of the active cankers was confirmed by isolating the fireblight organism from the extending margins. Of 24 varieties of apple observed, the following were found to overwinter the organism and are listed in the order of their importance in this respect in the situations studied: Transcendent, Hyslop, Yellow Transparent, McMahon, Wealthy, Fameuse, Tolman Sweet, McIntosh, and Dudley.

Overwintering in commercial apple orchards

Studies of overwintering of the blight organism in commercial orchards were initiated with the aim of gaining a clearer understanding of the types of lesion associated with carrying the bacteria through the winter, the host tissues in which overwintering ordinarily occurs, and the quantitative aspect of overwintering in relation to lesions on cankers, twigs and fruits.

Studies of cankers: Location of organism. On the basis of work done on pear, Jones (24) states that the blight organisms . . . "live over winter, not in the dead cankered tissue, but in the living bark immediately surrounding the cankered area." By studying cankers on apple during the late fall, winter, and early spring the present writer sought to gain a clearer conception of the relation of the overwintering organism to the tissues of this host.

During the fall and winter of the years 1923–24 and 1924–25 fireblight material was sent to Madison from the experimental orchards for isolation studies. This material consisted of blighted twigs ¼ to ½ inch in diameter, cankered branches ¾ to 2 inches in diameter with 8 to 12 inches of apparently healthy bark above and below the cankers, and mummified apples. Early in the spring of both 1924 and 1925 similar material was studied on the trees in the experimental orchards.

In all cases isolations were made by cutting away the outermost part of the bark with sterile scalpels, using a fresh scalpel for each cut, and then cutting out small pieces of the underlying tissues and treating them by one of the following methods:

(a) The material was ground fine in a sterile mortar in clean sterile quartz sand and sterile distilled water, and this mixture then plated in nutrient dextrose agar.

(b) The material was placed in a sterile petri dish, chopped fine with a sterile scalpel, mixed with ½ cc. of sterile distilled water, allowed to stand for 10 minutes, and then plated as just described.

(c) The material was placed in a tube of nutrient dextrose broth, chopped fine with a sterile scalpel in the neck of the tube, then mixed with the broth and incubated 24 to 48 hours at 28° C.

The pathogenicity of all cultures obtained was tested by pricking broth cultures or sterile distilled water suspensions of the organism with sterile needles into succulent twigs of Wealthy, Fameuse, or Transcendent trees.

From the cut branches sent to the laboratory, platings were made in some cases as soon as they were received, and in others after they had been kept for varying periods with their lower ends immersed in water. The cuts on the lower ends of the branches were made fresh from time to time, those at the upper ends were paraffined to prevent excessive drying, and the water in which the branches were placed was changed daily. The branches were kept in the 70° F. greenhouse until the buds had burst and the leaves had developed as far as they would under such conditions. It was thought that during this time the cankers might become active. Platings were made at various intervals during this period. Some of the branches were connected by means of rubber tubing to a hydrostatic pressure system so that the pressure of a 10-foot water column was applied to the lower cut surfaces. It was thought that this treatment might favor the production of exudate from the cankers. The results of all the above isolation experiments are given in table 1.

In Series III to VI, inclusive, attention was given to the type of margin of the canker, that is, whether it was cracked and well corked off or comparatively smooth and indefinite. It will be noticed that the greater number of isolations of *B. amylovorus* came from the smooth cankers. The organism was isolated in Series II from the live tissues adjacent to the margin of the canker; in Series III from the live tissues adjacent to the margin of the canker and from an area as far as ¾ inch beyond the margin; in Series IV from the margin of the discolored area of a smooth canker; in Series V from the extending margin of a canker on a branch which had been connected with the hydrostatic pressure system for 19 days; in Series VI from the margins and from the tissues up to one inch beyond the margins of smooth cankers. Of 277 cankers examined, 16 (5.7 per cent) were found to be holdover cankers. These figures are in general accord with data obtained by actual counts and observations of cankers in the orchards. Of 352 platings from the margins of cankers, 11 were positive. Of 417 platings from the area ¾ to 1¼ inches beyond the margins of the cankers, 19 were positive. All platings from the dead areas in the centers of the cankers gave negative results.

During the winter 1924–25, in addition to the isolations from cankers sent to the laboratory from the orchards, trials were made from cankers which had been produced upon a pear tree of unknown variety in the pathological garden at Madison by making scalpel incision inoculations into the large branches and trunk on August 13, 1924. Platings made from five of these cankers on February 11, 1925—five trials from the dead tissues in the

TABLE 1.—*Results of attempts to isolate B. amylovorus from apple cankers*

Series and dates of platings	Number and type of cankers[e]	Part of canker from which platings were made					
		Dead center		Margin		Living tissues ¾ to 1¼ inches beyond margin	
		Trials	Results[f]	Trials	Results[f]	Trials	Results[f]
		No.	No.	No.	No.	No.	No.
I[a] Nov. 6 to 28, 1923	25	50	50 –	50	50 –	50	50 –
II[a] Feb. 9 to 26, 1924	27	31	31 –	62	1 +, 61 –	64	64 –
III[b] May 13 to 18, 1924	65 C	65	65 –	75	75 –	75	75 –
	40 S	40	40 –	45	1 +, 44 –	55	2 +, 53 –
IV[c] Nov. 17 to Dec. 8, 1924	7 C	7	7 –	7	7 –	13	13 –
	18 S	18	18 –	18	1 +, 17 –	23	23 –
V[c] Feb. 24 to Mar. 15, 1925	24 C	24	24 –	24	24 –	38	38 –
	11 S	11	11 –	11	1 +, 10 –	23	2 +, 21 –
VI[d] Apr. 27 to 28, 1925	60 S	60	60 –	60	7 +, 53 –	85	15 +, 70 –

[a] Cankers on cut branches of Wealthy from Menomonie. Plated at Madison.
[b] Cankers on Wealthy at Menomonie. Plated at Menomonie.
[c] Cankers on cut branches of Fameuse from Gays Mills. Plated at Madison.
[d] Cankers on Fameuse at Gays Mills. Plated at Gays Mills.
[e] C = Cankers with margins well corked off. S = Cankers with smooth margins.
[f] – = *B. amylovorus* not isolated. + = *B. amylovorus* isolated.

centers of the cankers, seven from the margins, and eight from the tissues ¾ to 1¼ inches beyond the margins—gave two positive results. One of these was from tissues ¾ inch beyond the margin and the other from tissues one inch beyond the margin.

On February 14 a branch, 1½ inches in diameter, bearing two cankers was cut from the above mentioned pear tree and placed with the lower end in water in the greenhouse, where it was kept at 50° F. for 10 days, at 60° F. for a week, and then at 70° F. in an attempt to induce extension of the cankers. Before being brought into the greenhouse the cankers had been carefully outlined with a wax pencil. Under this treatment one of the cankers extended its boundaries. On March 17, as the buds were beginning to open, this canker had extended one inch beyond the lower margin, 1/4 inch above the upper margin, and 1/8 to 3/16 inch beyond the side margins.

TABLE 2.—*Results of experiments on the position of the fireblight bacteria in relation to apple cankers just renewing activity, Gays Mills, Wis., 1925*

Dates of culturing	Cankers tested		Results from cultures from stated locations[a]							
	Variety	No.	Dead center	Margin	Newly discolored area			Zone ¾ to 1¼ inches beyond newly discolored area		
					Outer bark	Inner bark	Outer wood	Outer bark	Inner bark	Outer wood
			No.	No.	No.	No.	No.	No.	No.	No.
Apr. 27	Hyslop	2	2−	2+	2+	2+	2+	2+	2−	2−
Apr. 28	Transcendent	3	3−	3+	3+	3+	2+, 1−	2+, 1−	3−	3−
Apr. 29	Transcendent	3	3−	3+	3+	3+	1+, 2−	3+	1+, 2−	3−
Apr. 30	Hyslop	3	3−	3+	3+	3+	3+	3+	3+	3−
May 16	Fameuse	5	5−	5+	5+	5+	2+, 3−	4+, 1−	1+, 4−	5−
May 18	Fameuse	5	5−	5+	5+	5+	4+, 1−	3+, 2−	2+, 3−	5−

[a] +=*B. amylovorus* isolated. −=*B. amylovorus* not isolated.

Platings were made from the dead central tissues and from these extending margins. No bacterial growth was obtained on the plates made from the dead tissues, but the plates made from the extending margins showed apparently pure cultures of bacteria which were proved by pathogenicity tests on young growing apples trees to be *B. amylovorus.*

The above experiments give further proof that the overwintering of the blight organism occurs, not in the dead tissues of the cankers, but in the apparently healthy tissues adjacent.

The isolations just reported were all made from material collected in a dormant condition. The following tests were made from cankers which had just resumed activity. By using cankers in which the organism was known to be present, and by making a greater number of trials from each canker, an attempt was made to secure more definite information as to the distribution of the organism in relation to the canker. The cultures were made by cutting away the tissues with sterile scalpels until the parts desired were uncovered without contamination, then cutting out a small piece with a sterile scalpel and placing it in a sterile tube of nutrient dextrose broth which was incubated for from 24 to 48 hours. The pathogenicity of these cultures was tested by pricking them into young apple twigs of Fameuse which had previously been protected by paper bags to insure their being free from blight. The results are given in table 2.

Here again the organism was not found in the dead tissues in the centers of the cankers but was isolated from the outer and inner bark of the extending margin in every case, and from the wood beneath the extending margin 13 times out of 21. From the area ¾ to 1¼ inches beyond the extending margin, it was isolated from the outer bark 17 times out of 21, and from the inner bark 7 times out of 21, but not from the wood beneath. These results give a gross conception of the relation of the organism to the host tissues during the progressive development of cankers. In the advancing zone the organism is present in the outer part of the bark, extending well beyond the macroscopically affected area, while in the later stages of development it progresses inward until it reaches the cambium.

Studies of cankers: The percentage of cankers overwintering B. amylovorus. Observations were made with the aim of securing more definite information as to the percentage of cankers which overwinter the blight organism. The results, which appear in table 3, show that the organism overwintered in sufficient abundance to furnish a plentiful source of inoculum for primary infection. The counts made on May 13 showed a much higher percentage of holdover cankers than usual due to the fact that the 100 cankers observed had been selected because of their smooth margins.

Studies of blighted twigs: Whether the blight organism is able, under Wisconsin conditions, to survive the winter in the small blighted twigs

remaining on the trees had not been definitely determined. Reinking (32), working upon this problem at the University of Wisconsin, was unable to isolate *B. amylovorus* from blighted twigs late in the fall, during the winter, or early in the spring. In Colorado, however, Sackett (33) was able to isolate the organism from as high as 21 to 33 per cent of blighted pear twigs. With these results in mind, the writer carried on the following work.

Platings made from blighted twigs brought to Madison from the orchards during the late fall and winter of both 1923 and 1924 all gave negative results, as did also those made in the early spring of 1924 from blighted twigs on Wealthy trees in the Weston Orchards. The platings were made from the basal portion of the dead tissues of the twigs and also from the live tissues immediately adjacent.

TABLE 3.—*The percentage of cankers overwintering B. amylovorus*

Place and date of observation	Variety	Trees No.	Cankers examined No.	Cankers active	
				No.	Per cent
Menomonie, Wis.					
July 22, 1924	Mixed	—	104	8	7.7
July 24, 1924	Wealthy	—	202	5	2.5
Gays Mills, Wis.					
May 13, 1925	Fameuse	30	100	11	11.0
May 19, 1925	do	35	160	13	8.1
May 21, 1925	do	145	291	16	5.1
do	Wealthy	55	25	1	4.0
May 22, 1925	do	100	129	6	4.6

It was not until the early spring of 1925 that observations in the orchards brought out the possible importance of small blighted twigs as holdovers of blight. On May 7 two McMahon twigs ¼ inch in diameter, which had been blighted the previous year, were found overwintering the organism. Three such twigs, 3/16, 1/4, and 3/8 inch in diameter, respectively, were found on a Hyslop crab; and three twigs, ½, ⅜, and ¼ inch in diameter, respectively, were found on a Transcendent crab. Each of these twigs had a drop of exudate on the live tissues immedately adjacent to the dead area.

On May 8, 9, and 10 several hundred trees and thousands of twigs which had been blighted the year before were examined to find what per-

centage of them overwintered the organism, as indicated by the production of the typical bacterial exudate. The results, which are given in table 4, show that overwintering of the organism in apple twigs may be of more importance than hitherto supposed. It is desirable that similar data be taken over a period of years and in other sections.

Studies of mummified apples. During the winters of 1923–24 and 1924–25 and the early spring of 1924 cultural studies of mummified apples clinging to blighted twigs failed to show the presence of *B. amylovorus* in any instance. The carpels of some of these apples contained a slimy ooze similar to the bacterial exudate produced by the blight organism, but all attempts to isolate the latter from this ooze gave negative results.

PRODUCTION OF BACTERIAL EXUDATE

Exudate is produced, under certain conditions, from all the blighted parts. In some cases exudation takes place even before the disease is macroscopically apparent in the tissues. In order to determine some of the factors influencing the production of exudate from blighted apple twigs, the following experiments were performed.

TABLE 4.—*The percentage of apple twigs blighted in 1924 which showed bacterial exudate, Gays Mills, Wis., May 8–10, 1925*

Variety	Trees	Blighted twigs observed	Blighted twigs bearing bacterial exudate	
	No.	No.	No.	Per cent
McMahon	360	5525	148	2.5
Fameuse	135	4300	28	0.6
Wealthy	250	1850	27	1.5

Eight Wealthy apple trees in 8-inch pots were used for each experiment, two being placed in each of four chambers (12) in which the temperatures were controlled. Two twigs on each tree were inoculated, and the points of inoculation were covered for 48 hours with cheesecloth wicks which dipped into small flasks of distilled water. The trees were placed in the chambers immediately after inoculation. Three sets of these inoculations were made on different dates. The rate of evaporation in the different chambers was the same and indicated relative humidities of 85 to 90 per cent in the 28° C. chamber, 80 to 85 per cent in the 24° C. chamber, 75 to 80 per cent in the 16° C. chamber, and 60 to 65 per cent in the 10° C. chamber. The results, which were taken after suitable incubation periods, are given in table 5.

While these data are too limited to be conclusive, they suggest that the optimum temperature for the production of exudate lies somewhere between 16° C. and 24° C. These results are in general agreement with those secured from the seasonal development studies of blight made in the orchards.

In 1925 the following sets of experiments were carried on in three controlled humidity chambers (22) the temperatures of which were 20° C. and the relative humidities, 50, 80, and 95 per cent, respectively. At the same time the relation of the vegetative vigor of the host plant to the production of exudate was studied.

On January 24, Wealthy trees were set in 8-inch pots, some in the ordinary potting soil and others in a mixture of potting soil and well rotted manure. They were kept in a greenhouse at approximately 60° F. On February 5 the buds started to burst. In order to secure excessively succulent growth some of the trees potted in soil and manure were treated at two week intervals with a nitrate solution.

TABLE 5.—*The relation of temperature to the production of bacterial exudate from blighted apple twigs, Madison, Wis., 1925*

Temperature in degrees C.	Production of exudate on twigs inoculated at stated dates		
	March 23	March 30	April 8
10	Little	Very little	None apparent
16	Abundant	Fairly abundant	Fairly abundant
24	Fairly abundant	Little	Little
28	None apparent	None apparent	None apparent

On March 16, two twigs of each of six trees were inoculated with a culture of *B. amylovorus* and the trees then placed in a moist chamber at 20–22° C. for 24 hours, after which time one of the trees which had received the special fertilizer treatment and one which had not were placed in each of the controlled humidity chambers. From time to time the production of exudate was noted. Final results (Series I) were taken on March 27.

On March 30 a similar experiment was performed. In this instance, however, instead of placing the trees for 24 hours in the moist chamber, the points of inoculation were covered for 48 hours with moist cheesecloth wicks which dipped into small flasks of distilled water. Final results (Series II) were taken on April 6. Results for both series are recorded in table 6.

From these experiments it appears that, although exudation takes place at all three humidities tested, it is most conspicuous at 80 per cent and is

more pronounced on exceedingly succulent twigs than on those of moderate growth. It is possible that at the highest humidity the exudate may have been rendered inconspicuous by taking up sufficient moisture to diffuse it.

In addition to these greenhouse experiments, observations upon exudate production were made in the experimental orchards. Records were kept of the days upon which exudation was observed. This record is given in Fig. 1, where it is correlated with other data. Before each period of exudate production there was a period of moist cloudy weather with moderately high temperature. Humidities above 80 per cent seemed most favorable for the production of exudate. Cloudy days followed by clear weather favored exudation. Probably one of the most important factors in the production of exudate is the condition of growth of the host plant. Just what this relation is has not been definitely determined.

TABLE 6.—*The relation of humidity and the condition of growth of the host plant to the production of bacterial exudate from blighted apple twigs, Madison, Wis., 1925*

Relative humidity in per cent	Production of exudate	
	From twigs with excessive growth	From twigs with ordinary growth
Series I		
50	Abundant	Little
80	Very abundant	Fairly abundant
95	Little	None apparent
Series II		
50	Fairly abundant	Little
80	Abundant	Fairly abundant
95	Little	None apparent

VIABILITY OF THE CAUSAL ORGANISM

Many investigators have worked upon the problem of the viability of *B. amylovorus* under various conditions, either in artificial culture or in naturally infected material. Gossard and Walton (17) found the organism to be viable in aphid honey dew 10 days after inoculation, and in honey for at least 72 hours. Fulton (16) showed that when blighted twigs were cut off during the period from July to late September and allowed to lie on the ground, nine-tenths of the twigs did not contain viable organisms after 7 days.

Under natural conditions *B. amylovorus* has been found by numerous workers to live over the winter in cankers and, in the present work, it has been shown to overwinter in blighted apple twigs even as small as 3/16 inch in diameter. In an endeavor to determine how long the organism remains viable in drops of exudate under varying conditions, the following experiments were performed.

Exudate exposed to direct sunlight. On June 27, 1925, fresh drops of naturally occurring exudate were collected from petioles, leaf-blades, small twigs, and small pieces of bark. These drops were exposed in open petri dishes to direct sunlight outside the field laboratory for varying lengths of time. After each of the different periods of exposure, 25 drops of the exudate were placed in tubes of nutrient dextrose broth, one drop in each tube, and incubated for 24 to 48 hours, at the end of which time the pathogenicity of the resulting cultures was tested on young blight-free Fameuse apple twigs, one twig being used to test each culture.

TABLE 7.—*The viability of B. amylovorus in drops of exudate exposed to direct sunlight, Gays Mills, Wis., 1925*

No. hours exudate exposed to direct sunlight	No. twigs inoculated	No. twigs blighted
½	25	25
1	25	25
3	25	25
6	25	25
9	25	21
15	25	10
22	25	2
30	25	0

The results which appear in table 7 show that the blight organism was killed in drops of exudate exposed to sunlight for a period of 30 hours. On the trees, however, where the exudate is not exposed to direct sunlight, the organism can probably live for a much longer time.

Exudate on cut twigs exposed to direct sunlight for two hours each day. On June 19, 1925, small twigs bearing abundant exudate were collected. After the leaf-blades had been cut off, these twigs were kept in wire baskets so that they were exposed to direct sunlight for only two hours each day.

After intervals of 7, 15, 25, and 35 days, cultures were made, each time from 25 drops of this exudate, and the pathogenicity tested as in the preceding experiment. Positive results were obtained after 7 and 15 days, but not after 25 and 35 days.

Exudate kept in wax paper bags. During July, 1924, large drops of exudate were collected, placed in wax paper bags, and kept in the laboratory. Nine months later the residues from these drops were cultured in broth, and the pathogenicity of these cultures tested. In no case was blight produced.

On May 19 and June 18, 1925, petioles bearing drops of exudate were placed in wax paper bags and pinned on the wall of the field laboratory so that they were open to the air but not in the direct sunlight. From time to time drops of this exudate were cultured and the pathogenicity of these cultures tested. Tests made 7, 20, 31, 40, 49, and 65 days after collection showed that the exudate still contained *B. amylovorus* in a viable condition. Lack of material prevented the tests being carried on for longer periods.

MODES OF DISSEMINATION OF THE CAUSAL ORGANISM

Though much work has been done on the modes of dissemination of *B. amylovorus,* additional studies of certain aspects of dissemination seemed desirable.

Meteoric water. Direct proof that rain is an active agent in the spread of fire-blight was first obtained by Gossard and Walton (17) in a series of experiments which were so controlled that rain was the only possible agency through which blight might be spread to the branches of the apple trees used for these tests. Stevens *et al* (34) conclude that the blight organism may be spread to a marked degree by the wind. Faulwetter (13) has demonstrated the manner in which *Bacterium malvacearum* may be disseminated by means of wind-blown rain, and suggests the possibility of other bacterial diseases of plants being spread in a similar manner (14).

In the present work, the part that meteoric water might play in the spread of blight was studied along two lines: (a) by observations in the orchards and (b) by infection experiments.

Owing to the very light infection of blight in the Weston Orchards during the season of 1924, no data could be secured upon this phase of the problem. However, observations made in the Kickapoo Orchards during the early development of blight in April and May of 1925 brought out the importance of rain as a factor in the spread of the disease. Even before much pink of the petals had been exposed in the blossom clusters, considerable blight had appeared on the young growing shoots, which at that time were from ½ to 1½ inches long. Upon close observation it was found that these infected shoots formed cone-shaped areas in the trees, and at the apices

of these cones were found actively exuding holdover cankers or twigs. Until about May 13, these infection cones could easily be traced to their sources of inoculum. Later they were partially obscured by the development of blossom blight. However, even some of the early blossom blight was found to be due to inoculum which was carried down by rain from exuding twigs above the blossom clusters.

Experiments were performed in order to determine whether infection from rain-borne inoculum could take place and under what conditions it would occur. The results indicate that rain can, under certain conditions, play an important part in the spread of blight. Dissemination of the blight organism over the individual tree, and to a limited extent from tree to tree, can be brought about by rain or heavy dews.

Insects. Ever since Waite (41) showed the relation of the honey bee to the spread of blossom blight, and Stewart (36, 37, 38) found that twig blight is spread through the agency of sucking insects, much interest has

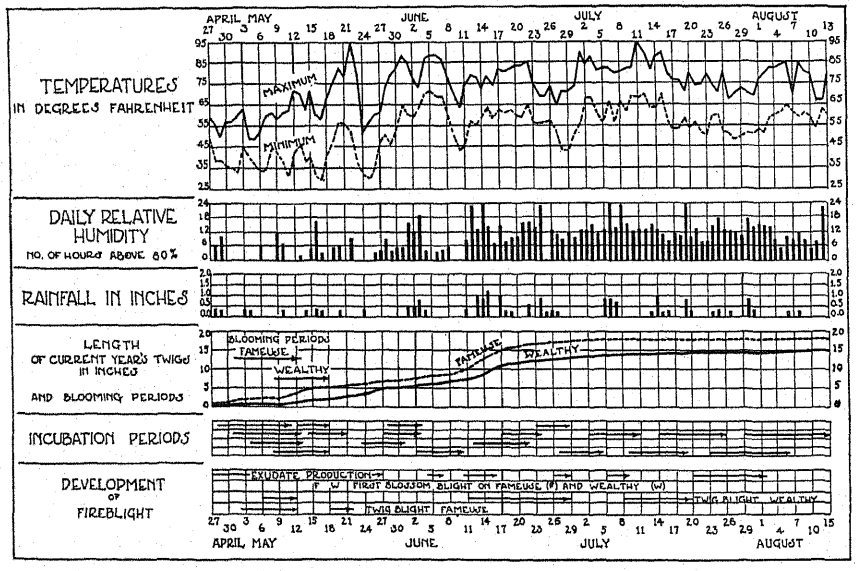

FIG. I.—Graphic summary of seasonal development and meteorological data, Gays Mills, Wis., 1925.

The meteorological data were taken in the experimental orchards by means of a hygrothermograph housed in a standard instrument shelter and a rain and snow guage of the type used by the U. S. Weather Bureau.

The length of current year's twigs is based on averages of records taken on fifty terminal shoots of each variety, representatively located on several trees.

The records on length of incubation period are based on puncture inoculations of current year's twigs of Fameuse and Wealthy.

been attached to insects as disseminators of fireblight. The list of offenders is a long one. Burrill (4) proved that aphids and leaf hoppers spread twig blight. B. J. Jones (23) listed many blight-disseminating insects, from flies to click beetles. D. H. Jones (25) showed the importance of the fruit-bark-boring beetle. Merrill (27, 28) further demonstrated that aphids are responsible for the spread of fireblight.

During the present work attention was given only to those insects which appeared in large numbers in the experimental orchards. In addition to the bees, the other important insects in disseminating blight were grain aphids, apple aphids, and leaf hoppers. In 1925 experimental and observational work was carried on with these two species of aphids and observations were made upon leaf hoppers to determine the extent to which these insects are responsible for the spread of twig blight. The results of this work, which appear below, accord with those reported by Burrill (4).

During the last week in April, 1925, the grain aphid, *Aphis avenae* Fab., was plentiful upon the apple trees. They not only covered the tips of the young growing shoots, but also travelled from place to place in the trees, crawling up and down the large branches and also upon the trunks. In their wanderings they traveled over exuding cankers and were observed many times to be caught in the bacterial exudate. In several instances the aphids were seen at the edge of the drops of exudate feeding upon it. It was noticed that the aphids feeding upon the drops of exudate remained there for several hours, but finally left it and went to other parts of the trees. Thus it is readily possible that aphids becoming contaminated in this way may bring about initial infection of spurs and twigs in the early spring. Examination of some of the blossoms just before they opened showed that aphids were present not only on the outside of the petals, calyces, and pedicels, but also inside the blossoms on the petals, filaments, styles, stigmata, and in the nectar. Since the aphids were found on exuding cankers and in the nectar of blossoms, it is possible that some of the initial infection of blossoms is brought about as a result of aphid-borne inoculum even before the blossoms are fully opened.

The results of the following experiment further strengthen the evidence that *A. avenae* can spread blight. On May 8, 10 young twigs of a Fameuse tree the tips of which were covered with *A. avenae* were inoculated with a suspension of *B. amylovorus* by means of a fine hypodermic needle. The inoculations were made about one inch below the tips at a point below the lowest feeding aphid. When the first symptoms of blight appeared, the twigs were cut off and the aphids transferred by means of camel-hair brushes to healthy twigs which had been enclosed in parchment paper bags for a sufficient period to ensure their being free from blight. Forty aphids were transferred from each of the ten twigs to healthy twigs, twenty aphids

to each twig. These healthy twigs bearing the aphids were then enclosed in celluloid cylinders as a protection against natural infection and also to protect the aphids against predatory insects, and were observed every day. Within 14 days all 20 of the twigs had blighted. As the twigs upon which the aphids are feeding become wilted from blight, the aphids commonly leave them and go to other young healthy shoots. This habit favors dissemination of twig blight.

An experiment similar to the above was performed on June 4. Five water sprouts of a Hyslop tree, the tips of which were covered with *Aphis pomi* De Geer, were inoculated by means of a hypodermic needle with a suspension of the blight organism as in the preceding experiment. As soon as the first symptoms of blight appeared, the twigs were cut off and the aphids transferred to healthy, blight-free water sprouts on Wealthy apple trees. These sprouts were enclosed in celluloid cylinders, and by June 20 all of them were blighted.

From the middle of June, 1925, to the middle of July, leaf hoppers were abundant on the apple trees. They caused the characteristic hopper injury to the leaves: the curling under and yellowing of the edges and a light brown specking of the under sides of the veins due to their feeding punctures. As blight appeared during this period, it was noticed in many cases that the disease first developed in the midribs of the leaves and from there proceeded down through the petiole into the twigs. From these observations it would appear that leaf hoppers are either capable of directly spreading blight or of so puncturing the leaves that infection may take place by means of rain-borne inoculum.

In Wisconsin, aphids and leaf hoppers appear to be the more important insects in the spread of twig blight. Click beetles do not occur in sufficiently large numbers to make them the menace they seem to be in California orchards (23) or in Oregon (8). The tarnished plant bug does not seem to be as plentiful in Wisconsin as it is in New York (36).

INFECTION STUDIES

Mode of entrance of causal organism. It is generally accepted that fire-blight infection of apple may take place through the nectaries of blossoms, but whether or not infection can take place through other natural openings, such as stomata and hydathodes of leaves, is still an open question. It was earlier thought that the blight organism gained entrance into leaves and twigs only through wounds, but in 1915 Heald (19) reported that probably stomatal and hydathodal infection takes place. In order to determine whether or not stomatal infection occurs under Wisconsin conditions, the following experiments were conducted.

The experiments of 1924 were performed in the orchard upon young Wealthy apple twigs which were apparently uninjured, although no attempt had been made to protect them previous to the time of inoculation. It was realized that, in this case, negative results as to stomatal infection would be valid, while positive results would not necessarily prove that stomatal infection had occurred, because there may have been microscopic injuries in twigs and leaves used. On June 30, a cloudy day, the upper and lower surfaces of the leaves on 30 young growing twigs were sprayed with a 48-hour-old broth culture of *B. amylovorus* diluted one-half with sterile distilled water. Ten of the twigs were enclosed in celluloid cylinders while the others were left exposed. By July 8 the apical leaf on each of five of the exposed twigs was blighted, but was not spotted as might be expected had stomatal infection taken place. When the final results were taken on July 23 no other twigs had developed blight.

On the evening of July 3 the leaves on 20 twigs similar to the above were inoculated in the manner just described, and none of them were enclosed. On July 12 the only blight evident was on the apical leaf of one twig.

Early in the morning of July 10, while wet with dew, the leaves of 40 twigs were inoculated as described above, and all the twigs were enclosed in heavy manila paper bags. The leaves of the twigs used had all expanded, but the terminal leaves were still immature. By July 23 none of these twigs had blighted.

During the winter of 1924–25 experiments were performed in the greenhouses in which potted trees were subjected to various environmental conditions before and after spraying the leaves with suspensions of the blight organism in an attempt to secure stomatal infection.

On March 9 the upper and lower surfaces of the leaves of four twigs on each of eight Wealthy trees which had been kept at approximately 70° F. were sprayed with a suspension of *B. amylovorus*. After inoculation the trees were kept in a moist chamber at 65–75° F. for 24 hours. They were then placed in the controlled temperature houses, two trees at each of the following temperatures: 50, 60, 70, and 80° F. The trees in the 70° and 80° F. houses did not develop blight. The tip of the youngest leaf on each of two twigs on trees kept at 50° F. and the youngest leaf on one twig of a tree kept at 60° F. became browned, and at 50° F. exudate formed on the petioles of the discolored leaves. In no case, however, was there definite indication of stomatal infection. The small percentage of infection which did take place may well have been due to microscopic wounds in the young leaves.

On March 16 a similar experiment was performed, using the leaves on two twigs of each of four trees. After inoculation and 24 hours in the the

moist chamber at 67° F., two trees were placed in the 60° F. house and two in the 70° F. house. None of the twigs developed blight.

In the next two experiments the trees were placed in the moist chamber at 65–75° F. for 36 hours before inoculation and for 24 hours after inoculation, in an attempt to have the stomata wide open and the substomatal cavities and intercellular spaces filled with moisture.

On March 11 three small Bartlett pear trees were taken from the moist chamber, and the upper and lower surfaces of the leaves of four twigs on each tree were sprayed with a suspension of *B. amylovorus*. The trees were replaced in the moist chamber for 24 hours, and then one tree was placed in each of the controlled temperature houses which ran at 60, 70, and 80° F., respectively. After five days the tips of the twigs on the trees kept at 60 and 70° F. showed slight browning, which later worked downward until the entire twigs were blighted. At 80° F. blighting did not occur so rapidly.

On March 11 an experiment parallel to the previous one was performed, using six Wealthy apple trees. The leaves on two twigs of each tree were inoculated and treated as described above, and two of the trees were placed in each of the controlled temperature houses at 50, 60, and 70° F. None of the leaves developed blight.

During the next growing season these experiments were resumed in the experimental orchard. The varieties used were Fameuse and Wealthy. Broth cultures of *B. amylovorus* diluted with water were sprayed only on the leaves of young twigs. Inoculations were made early in the morning, late in the evening, or on cloudy days, in the hope of catching the stomata when they were wide open. Before the time of inoculation about 50 per cent of the twigs used had been protected from natural infection by parchment paper bags. This method of protection was not always satisfactory because of the browning which took place at points where the leaves touched the paper. Consequently, half of the twigs chosen for the experiments had been unprotected previous to inoculation. They were, however, twigs which had grown rapidly during a rainy period in June and were apparently comparatively free from injury. Inoculations were made on May 20, 23, 27, 29, and June 2, 4, 12, and 15. After inoculations some of the twigs were enclosed in celluloid cylinders or paper bags, while some were left exposed. A summary of the results of these experiments is given in table 8.

None of the twigs which were protected from natural infection blighted; and of those not protected, eight twigs became completely blighted and seven had merely the tip leaves blighted. In no case was there evidence of stomatal infection.

In the above experiments the entire surfaces of the leaves were sprayed with the suspensions of the organism, but in the following tests the inoculum

was applied to small areas of the leaf surface in an attempt to minimize possible confusion incident to penetration of the organism through wounds. Circles ½ inch in diameter were painted on the lower surfaces of leaves with India ink and a camel-hair brush. A drop of the suspension of *B. amylovorus* was applied to the center of each circle by means of a camel-hair brush. Four or five of the youngest leaves on each twig were used. The inoculations were made on June 11, a cloudy day followed by another cloudy day with high humidity and two inches of rainfall. The twigs were observed daily but no blight developed. In view of the negative results of these and the preceding experiments it seems safe to conclude that under Wisconsin conditions stomatal infection does not commonly occur.

TABLE 8.—*Results of experiments on stomatal infection of apple leaves by B. amylovorus, Gays Mills, Wis., 1925*

Variety	Results on twigs which received stated treaments after inoculation					
	In celluloid cylinders		In paper bags		Exposed	
	No. inoculated	No. blighted	No. inoculated	No. blighted	No. inoculated	No. blighted
Fameuse	26	0	28	0	68	7
Wealthy	22	0	9	0	40	8

If stomatal infection does not take place, then to what extent must a leaf or stem be injured in order that rain-borne blight organisms may gain entrance and produce the disease, and for how long a time do these wounds remain active as infection courts?

In the Weston Orchards during the 1924 season it was observed that many of the leaves on the trees were injured by strong winds. There was the possibility that these injuries served as avenues of entrance for rain-borne inoculum. The following experiments were performed in an attempt to determine to what extent this was true.

Late in the afternoon of June 30, thirty twigs with naturally injured leaves were sprayed with a suspension of *B. amylovorus* and then covered with paper bags. By July 11 three of these twigs were blighted.

A similar inoculation of the leaves of 30 other twigs was made on July 11. The twigs were enclosed in paper bags after inoculation. Results taken July 26 showed that blight had developed in 10 of the twigs. These two experiments show that the blight organism may gain entrance into twigs through the wounds in leaves.

On July 6 the leaves on 30 twigs were stabbed with sterile scalpels so that the wounds were made through the intervenal areas and also through

some of the larger lateral veins. Immediately after wounding, the leaves were sprayed with a suspension of *B. amylovorus* and then covered with paper bags. Results taken July 18 showed that 27 of the twigs had blighted. From this experiment it was evident that fresh wounds on leaves may serve excellently as avenues of entrance for the blight organism. The next experiments were conducted to determine how long wounds serve as infection courts.

During the winter of 1924–25 experiments were conducted in the greenhouse. On March 10 the leaves of three twigs on each of six Transcendent trees were slit with sterile scalpels, cutting through the lateral veins. At intervals of 0, 2, 4, 8, 16, 24, 36, and 48 hours after injury, the leaves of two twigs were sprayed with suspensions of the blight organism. Each time the unsprayed twigs were carefully protected from the spray. After inoculation the twigs were enclosed in wax paper bags containing small wads of moist cotton. The results showed that blighting occurred in the twigs inoculated 0, 2, 4, 8, 16, 24, and 36 hours after injury, but not in the twigs inoculated 48 hours after injury.

On March 15 a similar experiment was performed, the inoculations being made 36, 48, 60, and 72 hours after injury to the leaves. Blight developed in the twigs inoculated 36 hours after injury, but not in those inoculated 48, 60, and 72 hours after injury.

On March 17 an experiment similar to the above was performed, using Wealthy apple trees. The results likewise showed that, up to 36 hours after injury to the leaves, the wounds served as infection courts for the fire-blight organism, but at the end of 48 and 60 hours these wounds were so changed that the organism did not gain entrance.

During May, two sets of the same type of experiment as the above were conducted in the orchard on Fameuse trees. The results showed that, after 30 hours, the wounds in the leaves no longer served as avenues of entrance for the blight organisms.

In the above experiments the larger veins of the leaves were injured, but in the following experiments the wounds were so made with the point of a scalpel or needle that none of the larger veins were injured.

On July 13, 1924, the laminas of the leaves on 30 twigs on Wealthy trees were punctured by needles so that none of the veins were injured. Immediately after injury the leaves were sprayed with a suspension of *B. amylovorus* and then enclosed in paper bags. By July 29 only one of the twigs was blighted.

On May 20, 1925, a similar experiment was performed, using the leaves on 25 twigs of Fameuse. No blight developed in any of the twigs.

Two further sets of this same type of experiment were conducted during June, 1925, upon the leaves of 25 twigs of Wealthy and 17 twigs of Fameuse.

Blight did not develop in the Wealthy twigs but did develop in one twig of Fameuse. This latter case may have been due to natural infection, since the Fameuse twigs were not protected previous to inoculation.

The results of the foregoing experiments tend to show that infection of leaves takes place more readily, if not exclusively, through wounds in the veins, and to a less extent, if at all, through wounds in the intervenal areas. Furthermore, these wounds in the leaves soon become altered in some way, possibly merely by drying out, so that they no longer serve as infection courts for *B. amylovorus*. In nature, of course, leaf infection very commonly results from passage of the blight organism from the subtending twig through the petiole.

Studies similar to the above were made upon injuries to stems of growing shoots. On July 14, 1924, the stems of 15 growing shoots of Wealthy apples were injured by pricking with fine sterile needles and immediately after injury were sprayed with a suspension of the blight organism and then enclosed in paper bags. By July 31 all of the twigs had blighted.

On May 27, 1925, the stems of 84 shoots of Fameuse were punctured each 20 times with fine sterile needles. At intervals of 0, 2, 4, 8, 19, 30, and 52 hours after injury, 12 of the stems were sprayed with a suspension of the blight organism. By July 16 all 84 of the twigs had blighted.

On June 4, 1925, the stems of 72 shoots of Fameuse were injured as described above; and at intervals of 48, 60, 72, and 84 hours after injury, 18 of the stems were sprayed with suspensions of *B. amylovorus*. By June 22 the twigs inoculated 48, 60, and 72 hours after injury had developed blight, but those inoculated 84 hours after injury had not.

On June 12 the stems of 100 twigs of Fameuse were punctured as in the preceding experiment; and at intervals of 24, 34, 44, 54, and 74 hours after injury, 20 of the twigs were sprayed with a suspension of the blight organism. By July 8 most of the twigs had developed blight, except those inoculated 74 hours after injury, of which only three had blighted.

The results of these experiments upon stem injury show that the wounds in the stems serve as points of entrance for the fireblight organism, but within approximately 74 hours after injury the wounds become so altered that they no longer serve as infection courts.

Factors influencing infection. That different varieties of apple vary in their resistance to fireblight has been observed by other workers. Among those who have published lists of varieties classified according to the susceptibility or resistance to blight are Anderson (2), Cate (8), Chambers (9), Green *et al.* (18), Hedrick and Howe (20), Hewitt (21), Norton (29) Stewart (35), Swingle (39), and Whetzel (43).

During the two year period, 1923–25, the relative susceptibility or resistance to fireblight of the different varieties of apple grown on a commercial

scale in Wisconsin was observed. In the following list these varieties are arranged according to their apparent susceptibility to blight under the conditions observed.

I. Very susceptible: Hyslop, Transcendent, Yellow Transparent, McMahon, Fameuse, and Wealthy.

II. Moderately susceptible: Tolman Sweet, Black Ben Davis, Jonathan, and Salome.

III. Moderately resistant: McIntosh, Duchess of Oldenburg, Grimes, Orange Winter, Wolf River, Whitney, and Stayman Winesap.

IV. Resistant: Ben Davis, Charlemoff, Delicious, Dudley, Northwestern Greening, and Tetofsky.

The Hyslop and Transcendent were the worst blighters observed. Large body cankers were found on trees of these varieties. Wealthy, one of the most widely planted varieties in Wisconsin, is very susceptible to twig blight, as is also the Fameuse. Dudley, in most cases, appeared to be fairly resistant. McIntosh had a small amount of twig blight which did not run down into the larger branches. Some spur blight resulting in the formation of small cankers appeared on the Duchess, but practically no twig blight or overwintering of the organism occurred on this variety. Delicious, Northwestern Greening, and Tetofsky seemed to be the most resistant of the varieties observed.

The exact nature of resistance of plants to fireblight has not been defined. It has, however, long been a matter of observation and record that plants in a high state of vegetative vigor are more susceptible to this disease than

TABLE 9.—*The relation of humidity and the condition of growth of the host plant to fireblight development, Madison, Wis., 1925*

Relative humidity in per cent	Development of blight			
	In twigs with excessive growth		In twigs with ordinary growth	
	Average length of blighted area	Average daily increase in length of blighted area	Average length of blighted area	Average daily increase in length of blighted area
Series I	Inches	Inches	Inches	Inches
50	5	0.42	2	0.18
80	11	1.00	5	0.42
95	9	0.82	4	0.36
Series II				
50	2	0.29	0.5	0.07
80	4	0.57	1.3	0.19
95	3	0.43	1.0	0.14

are those in low vigor. Greenhouse experiments substantiate these observations. Potted plants which had been forced into a vigorous, succulent growth blighted much more severely than did unfertilized trees which were in a lower state of vegetative vigor (Table 9).

In order to determine what influence temperature and humidity have upon the development of blight, the following experiments were performed. The methods of procedure with the controlled temperature experiments and those for the controlled humidity experiments have been explained above. From the results, which are given in table 9, it appears that blight develops more rapidly at the higher humidities, 80 per cent being slightly more favorable than 95 per cent. It also appears that the trees which had excessive succulent growth developed much more blight than did those which had not received the special fertilization treatment.

Series I of the temperature experiments reported in table 10 was conducted in the large controlled temperature greenhouses. The temperatures varied as much as two degrees from those given in the table. Series II was conducted in the small controlled temperature chambers where the temperatures did not vary more than one degree from those given. From these results it appears that the optimum temperature for the development of fireblight lies somewhere between 21° and 28° C. (70° and 82° F.).

SEASONAL DEVELOPMENT STUDIES

A summary of the records pertaining to the development of fireblight and of the apple plant for the season 1925 is given in figure 1, where they are correlated with certain meteorological data.

It will be observed that periods of exudate production were more dependent upon external environmental factors in the latter part of the season than they were early in the season. It may be that during the early part of the season the condition of the host is such that exudation will take place under a much wider range of external conditions than is the case later, when periods of high humidity, fairly high temperatures, cloudy days, and sufficient rainfall are necessary.

In 1925 the blighting of shoots occurred even before the appearance of blossom blight. In May, Fameuse had more twig blight than did Wealthy, but by the middle of June the situation was reversed, twig blight in the Fameuse being of small consequence while in the Wealthy it had occurred in epidemic form. This condition may be partly explained by the fact that the Wealthy had a much heavier bloom than did the Fameuse, and consequently, blight was more widely disseminated through the Wealthy blossoms which then served as foci for further infection of the rapidly growing twigs. From July 16 to August 4 there was little extension of blight in the twigs and but little new infection. After August 4 fireblight was apparently inactive.

TABLE 10.—*The relation of temperature to fireblight development, Madison, Wis., 1925*

Series I			
Date of inoculation	Temperature in degrees C.	Incubation period	Average daily increase in length of blighted area
		Days	Inches
Feb. 24	10	12	0.25
	16	8	0.37
	21	4	0.75
	27	4	0.63
Mar. 8	10	8	0.33
	16	6	0.50
	21	3	1.00
	27	3	1.13
Mar. 5	10	10	0.13
	16	5	0.17
	21	3	0.33
	27	2	0.50

Series II			
Date of inoculation	Temperature in degrees C.	Incubation period	Average daily increase in length of blighted area
		Days	Inches
Mar. 23	10	10	0.25
	16	6	0.66
	24	4	1.25
	28	2	1.13
Mar. 30	10	12	0.13
	16	7	0.50
	24	4	0.63
	28	4	0.63
Apr. 8	10	11	0.07
	16	5	0.38
	24	3	0.88
	28	3	0.75

Macroscopic symptoms of blossom blight did not appear until 13 days after the beginning of the blossoming period. The interval between the appearance of blossom blight on the Fameuse and on the Wealthy was about equal to the interval between the start of the blooming periods of the two varieties.

Artificial inoculations were made at frequent intervals to determine the incubation periods for twig blight throughout the season. The incubation periods of the earlier infections averaged about 13 days. As the season progressed they gradually shortened to a minimum of about 5 days during June, and then lengthened to a maximum of about 15 days in early August. This process, however, was not regular; there was considerable variation in the period of incubation without relation to the stage of the season. Incubation was hastened during periods of high humidity, rainfall, and fairly high temperature.

DEVELOPMENT OF EPIDEMICS

In Wisconsin, fireblight appears to some extent each year and frequently in epidemic form. In a given season it may be mild in its attack in one locality and severe in another. The direct loss to the apple growers is great only during epidemic outbreaks. It is important, therefore, to define as clearly as possible the chief factors which influence the development of epidemics. From the results of other investigators and those herein reported it appears that most of these factors center about weather conditions, disseminators of the disease, and the variety and condition of the host plant.

The present work has shown that the disease develops best at temperatures of about 65° to 85° F., and that epidemics are favored when such temperatures are accompanied by rains or high humidity. Rain, in addition to increasing the susceptibility of the plants by causing succulent growth, is also a direct disseminator of blight.

Since certain insects play a very important role in the spread of fireblight, the more numerous they are in the orchard the greater the chances for a blight epidemic. While the honey bee is perhaps the most important disseminator of blight, its work as a pollinating insect is indispensable in most apple orchards, since most commercial varieties of apple are self-sterile. The sucking insects, in addition to being able directly to transfer the blight organism from infected to healthy apple tissues, also wound the young growing parts so that the rain-borne inoculum can gain entrance.

Serious outbreaks of fireblight have frequently been occasioned through spread of the organism by careless use of pruning tools, or by inducing excessive growth of the host plant by injudicious pruning.

The susceptibility or resistance of the variety of apple has much to do with the overwintering of the blight organism and the subsequent develop-

ment of epidemics. Within a variety the condition of the host plant has an important influence on the extent to which blight will develop. Succulent growth is readily blighted, whereas a woodier growth is less easily invaded. Furthermore, from observations made over a period of three years, it appears that in Wisconsin epidemic outbreaks usually occur during the years of heavy bloom. The heavier the bloom, the greater are the chances for blossom blight to occur.

STUDIES OF METHODS OF CONTROL

Experiments on control measures were performed in the orchards along two lines: (a) the removal or treatment of blighted parts and (b) spraying for the control of insects which disseminate the disease. In 1924 the work was carried on in the Weston Orchards upon Wealthy, and in 1925 in the Kickapoo Orchards upon Wealthy and Fameuse. The trees were from 9 to 13 years old. The experimental plots contained from 75 to 300 trees each. Adequate check plots were left at various points in the orchards. These controls received no special treatment other than the regular lime-sulfur and lead arsenate sprays which were applied to the entire orchard for the control of apple scab.

Excision of blighted parts over small areas. During the first week in April, 1924, all of the blighted twigs and cankers were excised from 75 Wealthy apple trees in the Weston Orchards. The cuts were made 8 to 12 inches below the lowest evidence of blight. The tools and wounds were disinfected with Reimer's solution[2] and the larger wounds were covered with tree paint. When blight developed in the orchard during June, this plot showed very little disease; whereas in the check plots the blighting, though moderate, was about sixteen times as abundant. The following year, when blight appeared in epidemic form, this plot was practically free from the disease. The value of these data, however, is limited by the unusual sparseness of blight throughout the orchard in 1924. Furthermore, the apparently beneficial results noted in 1925 may have been due not only to the removal of blighted material but also to the fact that the trees in the plot thus treated did not bloom very heavily the second year.

During the first week in May, 1925, all blighted twigs and cankers were removed from a plot of 90 trees of Wealthy and Fameuse in the Kickapoo Orchards. On May 25, after blight had developed in that part of the orchard, a count showed that the disease had developed in five trees in the plot where excision was practiced, as compared with ten trees in the adjacent check plot. Later, however, after blossom blight had had a chance to appear, the amount of blight in the two plots was practically the same.

[2] One gram of mercuric chloride and one gram of mercuric cyanide in 1,000 cubic centimeters of water, as recommended through correspondence by Prof. F. C. Reimer.

This tends to indicate that the cutting out of blight in small areas may be effective in reducing the amount of the disease early in the sesaon, but that, with the opening of blossoms, blight may become so widely spread throughout the orchard by pollinating insects that the difference is obliterated.

Since it was found that many of the small blighted twigs may overwinter the organism and in some instances serve as more important sources of inoculum for initial infection than the larger cankers, on one plot only the blighted twigs were cut out, the cankers being left undisturbed. Observation, later, showed that activity of the organism had been resumed before the twigs were removed, and, as a result, infection had taken place in the twigs below. Consequently the results of this test were invalidated. This observation emphasizes the importance of early action when excision is employed. The work should be done either in the fall or well in advance of the opening of the apple buds.

Treatment of active cankers. In the spring of 1925, when an abundant supply of active cankers was available, different methods of treatment were applied to them. A sufficient number were left untreated to serve as checks. Notes were read only at times when the check cankers were actively exuding.

An aqueous solution of zinc chloride (20 per cent by weight) was painted over 25 cankers without removing the epidermis. This was twice the concentration suggested by Day (11). Three of these treated cankers were still active when examined one month later. One of the cankers of this series showed a clear amber exudate which tested negatively for the presence of *B. amylovorus*. These 25 cankers were on branches 1½ to 3 inches in diameter. In 13 cases, that part of the branch beyond the canker died after treatment.

In 25 cases the epidermis was scraped off with a sharp knife, not only from the cankers but also from the bark 3 to 4 inches beyond their margins. The wounds were painted with the following solution: 1 gm. mercuric chloride, 1 gm. mercuric cyanide, 500 cc. glycerine, 250 cc. 95 per cent alcohol, and 250 cc. water. Two cankers showed activity after treatment, due to the treatment not having included enough of the apparently healthy bark adjacent to the cankers. In only five cases did the part of the branch beyond the canker die under this treatment.

The epidermis was removed from 10 cankers including 8 inches above and below the cankers and 2 inches on each side. The wounds were painted with a mixture of carbol-fuchsin and gentian violet which, according to a report of a paper by Churchman (1), inhibits the reproduction of bacteria. None of the cankers so treated became active, and in only one case did the part of the branch beyond the canker die after treatment.

Briefly, the advantages of the last two methods reported above are that the activity of the bacteria was checked and but little time was required to

treat the cankers. The disadvantage is that much plant surface is left exposed to drying and to bark- and wood-rotting fungi. More work will have to be done upon these methods before they can be recommended for orchard practice.

Insect control. In the experimental orchards the chewing insects were combatted, as a part of the standard practice for the entire orchard, by the use of lead arsenate in the lime-sulfur sprays for scab.

On May 3, 1924, as the buds were bursting, three plots of 250 Wealthy trees each were treated with a miscible oil spray of a concentration of 1 gallon to 15 gallons of water. Good control of aphids was secured, and up to July 5 no blight had appeared on these plots. After that date a slight amount of blight appeared, carried in, most probably, by leaf hoppers from infected trees in the adjacent plots.

To control aphids further, nicotine sulfate spray (1 pint of a commercial nicotine sulphate solution to 100 gallons of water) containing soap or calcium caseinate as a spreader was applied to other plots as soon as the weather was warm enough to make the spray effective. On May 26, 1924, this spray was applied to 600 Wealthy trees, but the amount of blight in this plot in early June was about the same as that in the check plots. The spray had been applied too late to affect early blight infection.

The following year the aphids hatched out early in April (April 7–15), and a nicotine sulfate spray of the same concentration as in 1924 was applied to two plots of 240 trees each (120 Fameuse and 120 Wealthy) on April 14, when the temperature was well above 70° F. Another application of the spray was necessary on May 13, at which time the temperature reached 72° F. On May 25 there was an average of 0.7 blighted twigs per tree in the sprayed plots as compared with an average of 2.2 blighted twigs per tree in the check plots. Later, after blossom blight had appeared, the amount of blight in the sprayed and unsprayed plots was about the same.

From these results it appears that control of aphids reduces the chances of blight development early in the season before blossoming time. When there is a light bloom on the trees, control of aphids would probably do much to control blight in a given orchard even though neighboring orchards were infected. If the bloom is heavy, however, a blight epidemic may be started by the inoculum brought in from neighboring sources by pollinating insects. In such cases it seems necessary, if practicable, to remove the sources of inoculum from large areas.

In view of the fact that the greatest dissemination of blossom blight commonly occurs during the latter part of the blooming period, it was thought worth while to try to limit the spread of this phase of the disease through the use of bee-repellent sprays after sufficient pollination had been accomplished. Preliminary tests, however, showed that the material se-

lected for this purpose, a commercial repellent, failed to repel bees for more than a few minutes.

The present status of knowledge of fireblight does not appear to warrant confident recommendations of a satisfactory, practical method for the control of this disease under such climatic, cultural, and economic conditions as exist in Wisconsin. The most promising lines of attack appear to be timely excision of the blighted parts, insect control, and care in the selection of varieties and in cultural practice. The extent to which each of these methods is practical will vary with local conditions and remains to be determined by further investigation.

SUMMARY

Overwintering of *B. amylovorus* on apple in Wisconsin was found to occur in association with blighted twigs and cankers in the apparently healthy tissues adjacent to the dead areas. The organism was isolated from the tissues as far as one inch from the margins of dormant cankers. From 2.5 to 11 per cent of the cankers and 0.6 to 2.5 per cent of the blighted twigs studied in the spring of 1925 overwintered the organism. The size of twig does not limit overwintering since overwintering was found to occur in twigs as small as 3/16 inch in diameter. Hyslop, Transcendent, Yellow Transparent, McMahon, Fameuse, and Wealthy are the more important of the Wisconsin commercial varieties of apple in overwintering blight.

Most abundant production of bacterial exudate from blighted parts took place at temperatures between 65 and 85° F. and at relative humidities above 80 per cent. Alternate cloudy and sunny periods favored exudation.

B. amylovorus, when exposed in small drops of bacterial exudate to direct sunlight, was killed after an exposure of 22 hours. In drops of exudate kept in the laboratory in wax paper bags open to the air but protected from direct sunlight, the organism remained viable for more than two months and less than nine. In small blighted twigs cut from the trees and exposed to direct sunlight for two hours each day during June and July, the organism remained viable for 15 days but was dead at the end of 25 days.

Fully 90 per cent of the twig infection early in the season of 1925 could be traced directly to water-borne inoculum from exuding holdover cankers and twigs. Rain plays an important part in the local spread of blight throughout the season.

Aphis avenae, A. pomi, and *Empoasca mali* were observed to be the chief spreaders of twig blight in the experimental orchards. Aphids have been found feeding upon drops of exudate and also in the nectar of blossoms and may therefore be a factor in the initial infection of blossoms.

The nectaries of blossoms, and possibly the hydathodes of very young leaves, seem to be the only natural openings through which blight infection of apple takes place. All attempts to secure stomatal infection failed.

Wounds furnish the ordinary avenue of entrance to the bacteria for leaf and twig infection. Fresh wounds in the veins of young leaves serve as infection courts for water-borne inoculum. However, after some hours (36 to 48) these wounds no longer serve as points of entry for the blight organism. The same holds true for the wounds in stems except that these wounds may remain infection courts for as long as 72 hours. Pruning wounds on twigs may serve as avenues of entrance for *B. amylovorus* even during cold weather.

Of the varieties of apple grown on a commercial scale in Wisconsin, those more susceptible to blight are Yellow Transparent, McMahon, Fameuse, Wealthy, Transcendent, and Hyslop. Those showing a fair degree of resistance are Dudley, Duchess of Oldenburg, McIntosh, and Northwestern Greening.

The state of vegetative vigor of the host is a factor in determining the rapidity and extent to which blight develops. Even the more resistant varieties of apple will blight severely if too heavily fertilized and cultivated so as to produce an abnormal, succulent growth. It has also been noticed that blight epidemics tend to appear during the alternate years of heavy blossoming of the trees.

Factors favoring the development of epidemics of fireblight are temperatures from 65 to 85° F., high humidities, cloudy weather, frequent showers, infestations of aphids and leaf hoppers, an abundance of bees and other pollinating insects, the presence of large numbers of trees of the more susceptible varieties, heavy blossoming of the trees, and vigorous vegetative growth.

In the experimental plots the cutting out of blighted twigs and cankers over small areas during the dormant condition of the trees reduced the amount of blight developing early in the season; but, later, blossom blight was spread throughout these plots by pollinating insects which carried the disease in from surrounding infected plots.

Treatment of active cankers by merely painting them with a 20 per cent solution of zinc chloride was not satisfactory in checking their activity. The scraping of the epidermis from active cankers and adjacent bark and the application to the wound of Reimer's solution with the addition of glycerine and alcohol gave comparatively good results. The application of a mixture of carbol-fuchsin and gentian violet to active cankers from which the epidermis had been scraped checked the activity of the organism.

Spraying for the control of certain insect disseminators of blight checked the spread of the disease early in the season. These beneficial results disappeared, however, following the spread of blossom blight by pollinating insects. An attempt to mitigate this difficulty by using a bee-repellent spray after sufficient pollination had occurred was unsuccessful.

Further studies of fireblight are in progress at the Wisconsin Agricultural Experiment Station.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN.

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THE INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON WHITE ROT OF ALLIUM

J. C. WALKER

In a previous report (5) the appearance and distribution of the white rot disease of certain species of *Allium* (*Sclerotium cepivorum* Berk.) was discussed. Its widespread occurrence in western Europe generally is known, while its recent development in the United States has become a matter of immediate concern to the onion-growing industry. From a general survey of its distribution in Europe one is led to surmise that the disease is most destructive in the cooler portions of the growing season and that its progress is checked rather than enhanced by the warmer mid-summer weather. Likewise, it is of interest to note that the first establishment of the causal organism as a pathogen of economic significance in America was in eastern Virginia, where it attacked the winter-grown crop of multiplier onion. A third report of the disease in America was made in May, 1925, from Louisville, Kentucky (3).

Because of the imminent danger of the establishment of this disease in other onion-growing sections of the country, the writer became interested in undertaking a laboratory study of the influence of certain soil environmental factors upon the disease. At first, attention was given to soil temperature and soil moisture, and this paper deals primarily with the results of those studies.

A number of strains of *Sclerotium cepivorum* were collected. Most of them were isolated by the writer from collections made in Europe or from materials collected by others in America. Their several sources and the number by which they will be designated in this paper are as follows: No. 2, isolated from garlic collected in May, 1922, at Bañolas, Catalonia, Spain; No. 3, isolated from onion collected in June, 1922, in Brittany; No. 4, isolated from onion collected in June, 1922, on the island of Teneriffe, Canaries; No. 5, isolated from onion collected in September, 1922, on the island of Overflakkee, Holland; No. 6, isolated by H. H. Whetzel from multiplier onion or garlic collected at La Grange, Oregon; No. 7, forwarded by Professor Whetzel, who obtained the culture from Dr. Pethybridge in Ireland; No. 8, culture from Professor Whetzel, who isolated it from infected onions sent him by A. D. Cotton, Kew, England; No. 9, isolated from multiplier onion collected by Herbert Spencer, near Norfolk, Va.; No. 10, isolated from onion forwarded by W. D. Valleau from collections made

near Louisville, Kentucky. These strains behave very much alike in their growth upon potato dextrose agar. The greatest aberration in macroscopic appearance was noted in strain No. 9 which produced an unusually profuse aerial white mycelium which tended to obscure the sclerotia located upon the surface of the substrate. All of these strains, with the exception of No. 3, proved to be pathogenic to onion when introduced into soil in which the host plant was later grown.

TEMPERATURE STUDIES

The relation of temperature to growth of the cultures upon potato dextrose agar was studied. The cardinal points for growth were found to be very nearly the same for all forms. Very slight growth occurred at 4–5° C., and it was usually not macrsocopically visible within a fortnight. The most prompt development occurred between 20° and 24° C., and, on the basis of rate and amount of growth, the optimum was somewhere between these points. Although the rate diminished as the temperature was lowered, there was quite as much growth at 15° C. in a somewhat longer period. At 27° C. growth was distinctly retarded; strain No. 6 was more tolerant to this temperature than any of the other strains. At 28.5–29.5° C. only very meager growth occurred in most of the strains during a two-weeks period; strain No. 4 did not grow at all. At 32–34° C. none of the strains grew. The maximum temperature for growth on potato dextrose agar therefore, was, in the neighborhood of 29° C., the minimum somewhere below 5° C. while the optimum was around 20° to 24° C. With the exception of a slightly greater tolerance to higher temperatures by strain No. 6, the various cultures reacted quite similarly to the various temperatures. The results of a single experiment are illustrated in Plate XXXV, A.

The obviously important question is that of the relation of temperature to infection and to the progress of the disease. Since the subterranean parts of the host are the ones first subject to invasion, the soil temperature relation is one of primary interest. Because of the similarity of the strains in their temperature reactions on culture media, most of the further work was conducted with a single strain, No. 5 from Holland having been selected. The sclerotia from pure cultures were mixed with virgin soil and several crops of onions grown until it was certain that the soil was thoroughly infested. Pot cultures were then started in galvanized iron cylindrical pots which were held at various desired temperatures in Wisconsin temperature tanks. Onion seedlings, grown to the third or fourth leaf stage, or onion sets were then planted in the soil. In certain cases bulbs or seedlings of other species of *Allium* as well were used in the experiment. The soil moisture in these series was adjusted so as to support good growth of the host and was kept reasonably constant by frequent weighing of the pots and replacement of lost water.

TABLE 1.—*Development of white rot in Crystal Wax Bermuda onion seedlings held at various soil temperatures*

Soil temperature in degrees C.	Inoculated soil					Uninoculated soil		
	No. plants used	After 23 days		After 40 days		No. plants used	After 40 days	
		No. healthy	No. diseased	No. healthy	No. diseased		No. healthy	No. diseased
14	10	0	10	0	10	5	5	0
18	10	0	10	0	10	5	5	0
22	10	7	3	5	5	5	5	0
24	10	8	2	5	5	5	5	0
26	10	10	0	10	0	5	5	0
30	10	10	0	10	0	5	5	0

Crystal Wax Bermuda seedlings in which four true leaves had emerged, were used in the first experiment. These were transplanted to pots of inoculated soil, and a certain number were transferred to pots of uninoculated soil. They were then placed in tanks which were kept at various constant temperatures. The air temperature of the greenhouse varied from 15° to 20° C. Although the temperatures of the water bath in the tanks varied less than one degree above or below the desired point, the soil at the surface of the pots, not being protected from the influence of the air temperature, varied somewhat more than this. The temperature of the surface inch of soil in the pots held at 14° C. therefore tended to run somewhat higher than the remainder of the pot, while in the case of pots kept at temperatures above that of the air the surface soil tended to run somewhat lower than the average for a given pot. Since the infection of the host takes place chiefly in the upper part of the pots, this error is to be considered in interpreting the final data. The disease appeared in the 14°, 18°, and 22° C. tanks on or before the thirteenth day. The results at the end of 23 days and 40 days are given in table 1.

The disease developed most rapidly at 14° and 18° C. At the end of the 40-day period all plants in these tanks were dead. At the two intermediate temperatures, 22° and 24° C., 50 per cent or less of the plants were affected, and not all of the diseased plants had succumbed at the end of 40 days. At 26° and 30° C. no infection occurred.

A second experiment of similar nature was conducted in which seedlings of three varieties of onion were used. Since the tops of the pots were not protected, the discrepancy in temperature between the upper layer of soil and the remainder of the pot still obtained. The limits of variation were therefore recorded. The results at the end of 80 days are recorded in table 2.

Infection proceeded equally well in each of the three varieties, but the influence of temperature was again evident. The progress was greatest at the lower temperatures, as in the previous experiment. At 23° C. and above there was again a decided limitation in infection. Representative pots of Red Globe variety in inoculated and uninoculated soil at different soil temperatures are illustrated in Plate XXXIV.

In order to eliminate the variation in the soil temperature at the top of the pot which prevailed in the experiments just described. two more series were conducted in which this factor was largely controlled. This was done by covering the soil with a layer of mineral wool as soon as the plantings were made, a procedure which served to exclude the influence of the air temperature upon the soil, and provided a more nearly uniform soil temperature throughout the pots. The soil was thoroughly mixed before potting so as to insure uniform distribution of moisture. As in previous

TABLE 2.—*Development of white rot in seedlings of Red Globe, White Globe and Crystal Wax onions at various soil temperatures; duration of experiment, 80 days; air temperature, 20–25° C.*

Soil temperature in degrees C.	Temperature range in degrees C. in upper layers of soil	Inoculated soil						Uninoculated soil			
		Red Globe		White Globe		Crystal Wax		Red Globe		Crystal Wax	
		No. of plants	No. diseased	No. of plants	No. diseased	No. of plants	No. diseased	No. of plants	No. diseased	No. of plants	No. diseased
10	10–11	14	14	18	18	18	18	9	0	9	0
14	14–15	18	17	18	15	18	18	9	0	9	0
18	18	18	18	18	18	18	18	9	0	9	0
22	20–22	18	10	18	15	18	16	9	0	9	0
26	23–26	18	1	18	1	18	1	9	0	9	0
30	25–30	17	0	17	1?	17	0	8	0	7	0

experiments, the moisture was kept approximately constant during the course of each experiment by frequent weighing of each pot and replacement of lost water. In the third experiment Crystal Wax Bermuda seedlings were used, and the air temperature was somewhat higher than in the other experiments reported. In the fourth experiment the observations were extended to certain other susceptible species of *Allium*. Bulbs of garlic (*Allium sativum*), white multiplier onions (*Allium cepa* var. *multiplicans*), White Portugal onion (*Allium cepa*), and seedlings of White Welch onion (*Allium fistulosum*) were used. The final data collected are recorded in tables 3 and 4.

With the maintenance of uniform temperature throughout the pots, more critical judgment of the aggressiveness of attack at various points in the temperature range was possible. In the third experiment the disease seemed to develop with nearly equal severity from 10° to 20° C. (see table 3). If there was any variation within this range it would appear that the progress and destructiveness of the organism was somewhat greater at 16° C. than at temperatures above or below that point. With the more accurate control of temperature the exact point at which infection is inhibited becomes more apparent. Comparing the evidence at 20° and 22° C. it is to be seen that at the former temperature early infection occurred and all of the plants succumbed, while at the slightly higher temperature the appearance of the disease symptoms was not only delayed but the infection was confined to only one of ten plants. Constant temperatures of 24° C. and above were sufficient to prevent invasion by the parasite.

In the fourth experiment (table 4) the reactions of garlic, Welch onion, multiplier onion, and common onion were studied. The largest percentages of infection and the greatest advance of the disease were found at 14° and 18° C., showing that variation in host plant had no apparent bearing on the temperature relation. The 8–10° C. series was lower than any maintained in previous experiments, and inhibition of infection at these temperatures was evident with all four host plants. The absence of disease above 22° C. was noteworthy in each species of *Allium* tested. The reduced percentage of infection in the case of the multiplier onion is significant and suggests the possibility of variation of susceptibilty among known host species or varieties. Cotton and Owen (1) have already recorded some observations along this line.

The temperature reaction of a parasitic fungus as indicated by its vegetative growth on a favorable culture medium is not always a safe criterion for predicting the relation of temperature to its pathogenic properties. In this case the discrepancy is noteworthy. Although the greatest amount of vegetative growth on potato dextrose agar in a given period was above 20° C., infection was greatly retarded beyond this point. Nearly as good

TABLE 3.—*Development of white rot in Crystal Wax Bermuda onion seedlings at various soil temperatures; air temperature 15–20° C.*

Soil temperature in degrees C.	Inoculated soil					Uninoculated soil		
	No. of plants used	After 26 days		After 40 days		No. of plants used	After 40 days	
		No. healthy	No. diseased	No. healthy	No. diseased		No. healthy	No. diseased
10	10	2	8	1	9	10	10	0
13	10	3	7	0	10	9	9	0
16	10	0	10	0	10	10	10	0
18	10	4	6	2	8	10	10	0
20	10	2	8	0	10	9	9	0
22	10	10	0	9	1	9	9	0
24	9	9	0	7[a]	0	10	10	0
26	9	9	0	9	0	8	8	0
28	9	9	0	9	0	8	8	0
30	9	9	0	9	0	9	9	0

[a] Two died from other causes.

TABLE 4.—*Development of white rot in garlic, white multiplier, White Portugal, and White Welch onions at various soil temperatures. Duration of experiment 73 days*

Soil temperature in degrees C.	Garlic		White Multiplier		White Welch		White Portugal	
	Total no. plants	No. diseased	Total no. plants	No. diseased	Total no. plants	No. diseased	Total no. plants	No. diseased
8–10	8	0	9	2	10	3	10	7
14	8	7	10	2	10	7	10	10
18	8	6	9	4	10	9	10	10
20–22	10	1	8	1	10	2	10	3
26	9	0	5	0	10	0	10	0
28–30	10	0	10	0	10	0	10	0

growth was secured between 15° and 20° C. after a somewhat longer time had elapsed. The fungus is a very active pathogen, however, from 10° to 20° C. There appears to be little connection between rate of growth on potato dextrose agar and pathogenicity.

In this connection it may be well to consider the influence of soil temperature upon the development of the host. Some earlier data were given on the growth of young seedlings of Red Globe and Yellow Bermuda onions during the first 30 days after sowing (6). In the Red Globe (a northern variety) the best development of roots was found at soil temperatures below 20° C., while the best development of tops was at 20° C. and above. In the Yellow Bermuda (a southern variety) the best root growth was at 20° C. and the best top growth at 25° C. In the first soil temperature experiment reported in this paper the control plants in uninoculated soil were carefully washed out of the soil after 52 days and the dry weights of the roots and tops determined. At 22° C. and above the plants had formed distinct bulbs, while at 14° and 18° C. the leaf bases were just starting to thicken. The bulb and the remainder of the top were dried separately in each case. At the same time a series of plants from White Portugal sets was run for a slightly shorter period in uninoculated soil. The dry weights of these were likewise procured. In table 5 the results of the two lots are summarized. The best root development, as in the young seedlings, was at 22° C. or below while in general the best bulb development was at

TABLE 5.—*Dry weights of roots, bulbs, and leaves of White Portugal and Crystal Wax Bermuda onions held at various constant soil temperatures, but exposed to the same air temperature (15–20° C.)*

Variety, condition, and dates	Soil temperature °C.	Av. wt. of roots (grams)	Av. wt. of bulbs (grams)	Av. wt. of leaves (grams)	Av. wt. of bulbs and leaves (grams)	Av. wt. of total plants (grams)
White Portugal plants started from sets. April 16 to May 24.	10	.307	.713	1.196	1.909	2.216
	14	.250	.947	1.257	2.204	2.454
	18	.198	1.365	1.692	3.057	3.255
	22	.283	1.649	1.678	3.327	3.610
	26	.099	1.527	1.054	2.581	2.680
	30	.064	1.341	1.227	2.568	2.632
Crystal Wax Bermuda seedlings in 4th leaf and about 6 inches high at time of transplanting to pots. April 14 to June 5.	14	.118	.145	.508	.652	.770
	18	.135	.286	.525	.810	.945
	22	.100	.615	.546	1.160	1.260
	24	.066	.843	.288	1.131	1.197
	26	.040	.637	.117	.754	.794
	30	.018	.734	.080	.814	.832

22° C. and above. As a whole the most rapid growth occurred in both varieties at 22° C. The critical point, then, at which infection is abruptly inhibited is not that at which there is an evident check in the growth of the fungus in pure culture, but that at which the host is growing most vigorously. One may be led to question, therefore, whether or not the influence of temperature upon the host metabolism may not have an indirect bearing upon the relation between host and parasite.

SOIL MOISTURE RELATIONS

In the foregoing experiments the influence of temperature was the primary consideration. In each case the soil moisture was approximately the same throughout the experiment, but no attention was given to the influence of different amounts of soil moisture upon infection. The next experiment was planned in order to study the latter question. The same soil was used as in previous experiments, and division into four lots was made. The water-holding capacity of the soil was found to be 60 per cent of the dry weight. The four lots of soil were then adjusted respectively to moisture contents approximating 80, 60, 40, and 20 per cent of the water-holding capacity. The soil was placed in galvanized iron cylindrical pots 5 inches in diameter and with a depth of soil of about 5 inches. Three pots were made up for each soil moisture. The pots were then planted with White Portugal sets and placed in a greenhouse at a temperature of 15–20° C., which was within the range at which best development of the disease had previously been found to occur. The pots were weighed daily and sufficient water added to bring each up to the original weight. This method of maintaining soil moisture is of course subject to some error, and variation within the pot is to be expected. It is practically impossible to compensate accurately for the increase in weight due to the growth of the plants, so no attempt was made to correct this error. As a result, when the soil moistures were determined at the end of the experiment, it was found that the moisture content had been reduced somewhat in the wetter soil where the greatest amount of growth occurred.

The experiment was made over a period of 48 days. The soil moistures at the beginning and end of the period, together with the amount of disease, are given in table 6.

The lowest moisture content, 13 per cent, was not sufficient to promote anything but very meager growth and was entirely too low for normal development of the plant. Infection also was apparently retarded by the lack of sufficient moisture. The top growth of the onions increased with the soil moisture, and the rankest leaf development occurred in the highest soil moisture. The plants grew well at 23 per cent moisture content, but infection occurred here first and the disease progressed most rapidly. At

TABLE 6.—*Relation of soil moisture to development of white rot in White Portugal onions; duration of experiment, 48 days; water-holding capacity of the soil, 60 per cent*

Lot No.	Moisture content of soil in percentage		Condition of plants at end of experiment		
	Beginning of experiment	End of experiment	Total no. plants used	No. healthy	No. diseased
1	13	12	24	12	12
2	23	26	24	0	24
3	37	30	24	19	5
4	48	37	22	20	1

the conclusion of the experiment all of the plants at this temperature were affected and most of them were dead.

The behavior of the plants at the higher soil moistures is also noteworthy. In lot 3, where the soil moisture was kept within 50 to 60 per cent of the water-holding capacity, 20 per cent of the plants were diseased as compared with 100 per cent in the soil with a moisture content of 23 per cent. In lot 4, where soil moisture was raised to a still higher point, the amount of infection was still less. Representative pots from each lot are illustrated in Plate XXXV, B.

The healthy plants were just beginning to bulb in lots 3 and 4 at 48 days. The moisture of the soil was raised again to 37 and 48 per cent respectively, the pots being kept at constant weights as before, and most of the plants were allowed to continue growth for another 53 days. By that time bulbs had formed averaging about one inch in diameter. One more plant in lot 3 had succumbed to white rot during that period, while in lot 4 one plant was infected at the neck of the bulb. All the remaining plants were apparently sound.

It is thus evident that the amount of moisture in the soil may greatly affect the severity of the disease even at the optimum temperature. It is interesting to note that within the temperature range favorable to growth of the plant, the fungus becomes less destructive as the rapidity of host growth increases. There remains for further investigation the question of whether this shift in soil environment constitutes a direct retardation of the parasite or a stimulation of greater resistance as a result of metabolic changes in the host, or both.

DISCUSSION

The data which have been presented establish the fact that soil temperature has a marked influence upon the development of white rot. The latter

may be properly classed as a low temperature disease, and its greatest destruction may be looked for when the average soil temperature is 20° C., or below; while at temperatures above 22° C. the host may be expected to escape infection. In addition to temperature, the soil moisture is equally important. The disease may be expected to occur at as low soil moisture as will support growth of the plant, while in soil where the moisture is above 50 per cent of the water-holding capacity a gradual reduction in infection will occur as the moisture is raised even though the temperature is at the optimum for the pathogenicity of the organism. Just how the influence of these soil environmental factors is exerted upon the parasitic relation remains an unanswered question. It is pointed out that the optimum temperature for growth of the fungus on potato dextrose agar lies in a range where little infection occurs. Is there an optimum temperature for vegetative growth on potato dextrose agar and another for the expression of its pathogenic properties in the soil habitat? Or is the metabolism of the host at the higher temperatures the limiting factor? In the same way, are we to attribute the reduction of the disease in the wetter soil to inhibition of the parasite or to stimulation of the host?

On the questions of general distribution and destructiveness of the disease, the facts have an important practical bearing. The prevailing soil moisture is likely to be extremely variable throughout the season in a given locality, and it is quite probable that such variations have their effects on the development of the disease. With regard to soil temperature it is easier to predict that certain localities where onions are grown offer more favorable conditions than others. For instance, in the northern onion-growing sections of the United States the crop is subjected to ideal temperature conditions for white rot during the early part of the growing season while following midseason the soil temperature is likely to be quite unfavorable. Such records as have been accumulated for southeastern Wisconsin (4, 5), where onion sowing is done, as a rule, in April, show that during May and part of June the daily mean temperature of the upper 2 inches of soil is usually below 20° C., while for the rest of the onion growing season it is usually above 20° C. In sections in the tier of states south of Wisconsin the period of favorable temperature is probably somewhat shorter. When one considers still more southerly sections, however, where the seed is commonly sown or sets planted in the autumn, it is quite likely that a longer period of favorable soil temperature prevails. Moreover in this region white rot has already become an established disease.

SUMMARY

The growth of the white rot organism (*Sclerotium cepivorum* Berk.) on potato dextrose agar occurs from somewhat below 5° C. to about 29° C. The

most rapid development takes place between 20° and 24° C. while it is nearly as great at 15° to 20° C. after a somewhat longer period of time.

Infection occurs readily and the disease progresses most rapidly at soil temperatures between 10° and 20° C. At 20° and 22° C. there is a marked reduction in infection, and at constant temperatures of 24° C., or above, the disease does not appear. This temperature reaction holds for three varieties of common onion; multiplier onion, Welch onion, and garlic.

In the soil used, the greatest development of the disease occurred when the soil moisture was kept at about 40 per cent of the water-holding capacity. At 20 per cent of the water-holding capacity the growth of the host was very slow and the amount of disease was reduced. At 60 per cent and 80 per cent of water-holding capacity the growth of the onion plant was accelerated but infection was greatly reduced.

It is pointed out that, under natural conditions, variations in soil moisture may have a direct effect upon the occurrence of the disease. In the northern onion-growing sections of the United States the most favorable soil temperatures occur during the first half of the growing season while during the last half the daily mean soil temperature for the upper layers of soil is, for the most part, above the point most favorable for disease development (10°–20° C.). In the more southerly sections, where winter crops of onion and related plants are commonly grown, the favorable temperatures probably extend over a long portion of the growing season.

OFFICE OF VEGETABLE AND FORAGE DISEASES,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
and
DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN.

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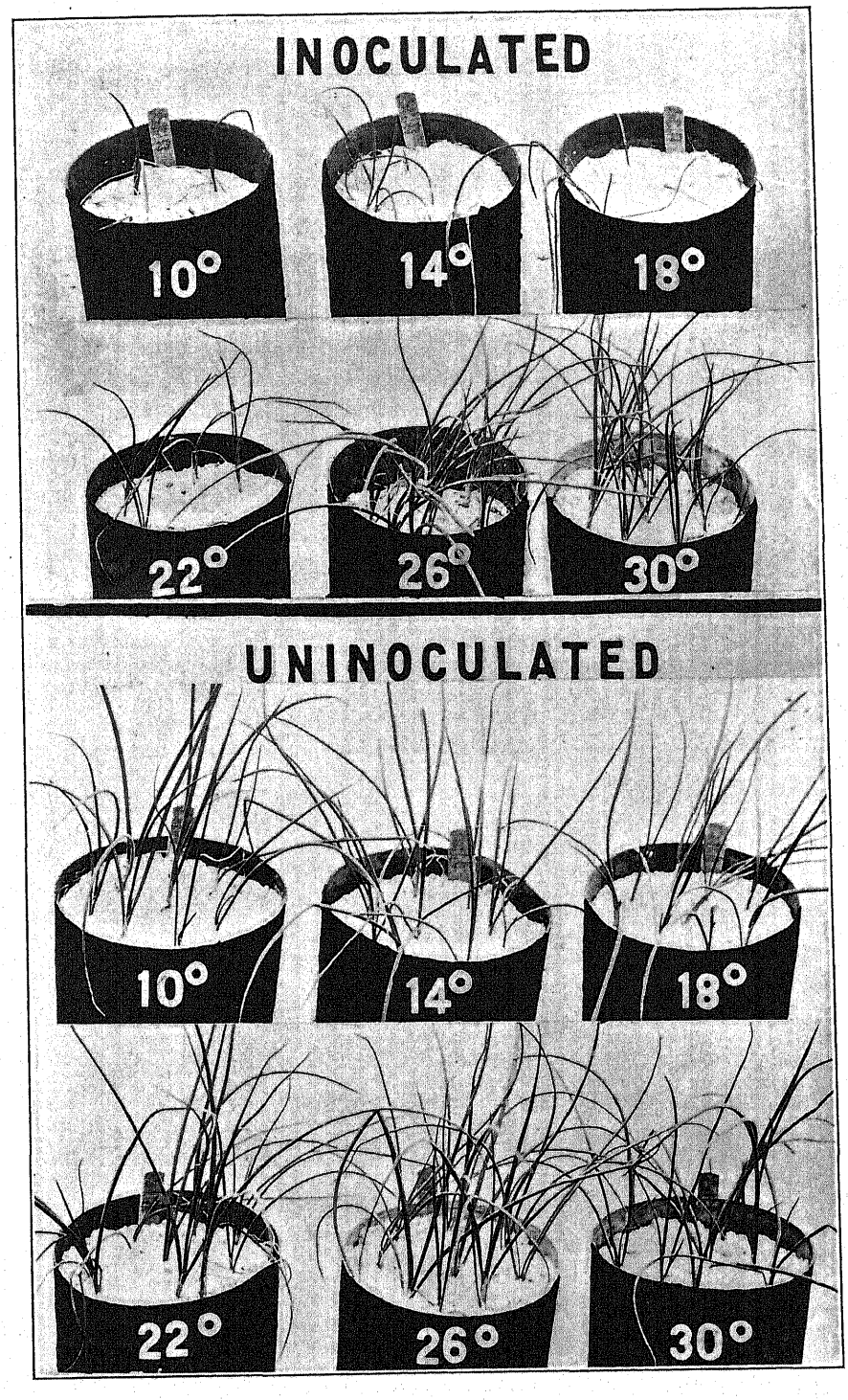
EXPLANATION OF PLATES

Plate XXXIV. Red Globe onion seedlings 40 days after being transplanted to soil inoculated with *Sclerotium cepivorum* and to uninoculated soil. The soil was held at the temperatures indicated, and the air temperature varied from 15° to 20° C. Since the tops of the pots were not covered in this experiment, there was more variation in the upper layers of soil than in later experiments. At this stage the fungus had destroyed most of the plants at 10°, 14°, and 18° C. soil temperature, while in the 22° C. tank, where the upper soil varied between 20° and 22° C., the progress of the disease was not so great. At 26° and 30° C. the plants were free from infection. In subsequent experiments in which the soil was covered with mineral wool and more uniform temperature maintained throughout the pot no disease occurred above 22° C.

Plate XXXV. A. Growth of *Sclerotium cepivorum* on potato dextrose agar plates after a period of 16 days at the various temperatures indicated.

B. Onions grown from sets planted in soil inoculated with *Sclerotium cepivorum* and held at various degrees of soil moisture for 48 days. The water-holding capacity of the soil was 60 per cent of the dry weight. The moisture content of the soil in the pots was as follows: (1) 12–13 per cent. (2) 23–26 per cent. (3)30–37 per cent. (4) 37–48 per cent.

In lot 1, where there was insufficient moisture to support good growth, 50 per cent of the plants were diseased; in lot 2, 100 per cent were diseased; in lot 3, 20 per cent; in lot 4, 5 per cent.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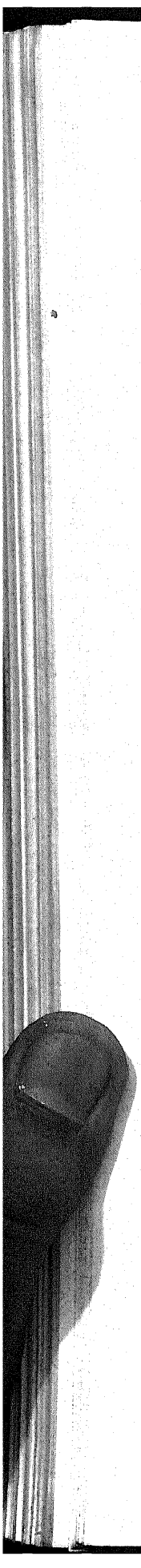

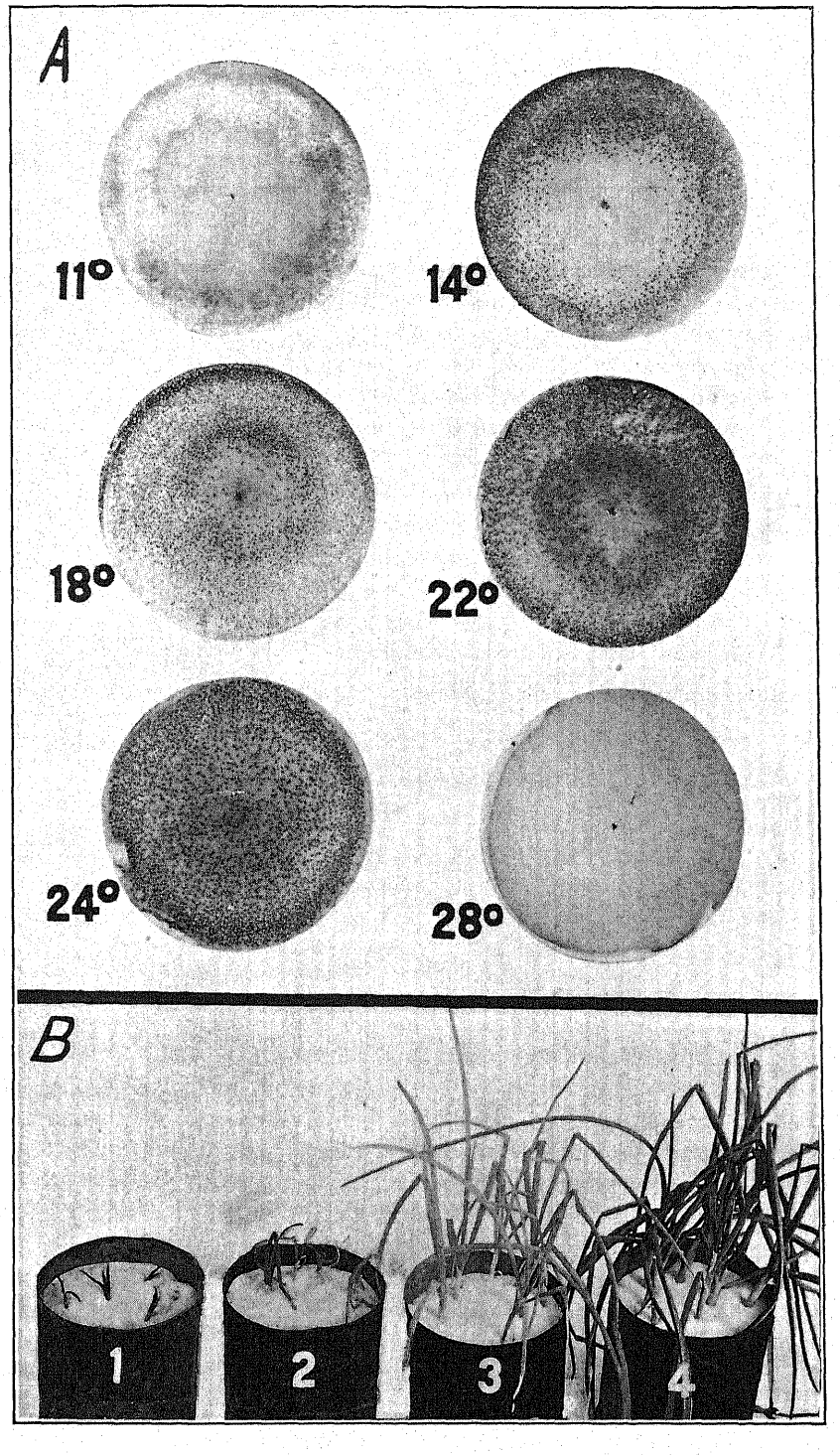
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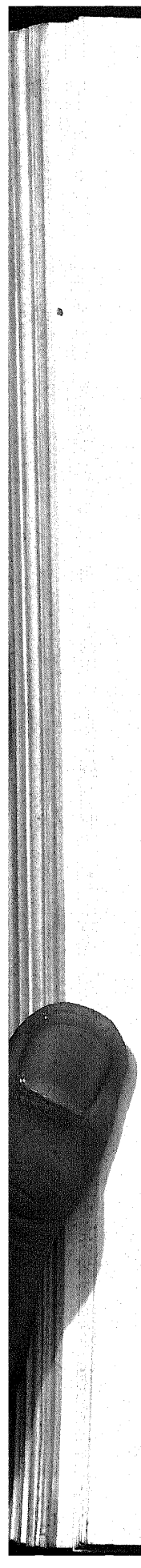

SPRAIN OR INTERNAL BROWN SPOT OF POTATOES

D. ATANASOFF

COMMON NAMES

In Holland, sprain or internal brown spot of potatoes is generally known under the name "Kringerigheid" (ringiness). In the German speaking countries the disease is known under a large variety of names. Of these "Eisen" (50) and "Buntfleckigkeit" are perhaps most commonly used. But the disease is also known under the following names: Buntwerden der Kartoffeln, Pfropfenkrankheit, Braunfleckigkeit (59), Rostfleckigkeit, Ringbräune, etc.

In England the same disease is commonly called "sprain" (37). In 1910 Horne (19) distinguished two forms of this disease: "internal disease" and "sprain" or "streak" disease; and, in 1914 (20), "blotch" and "streak." Paine in 1918 (33) and 1923 (34) used the name "internal rust spot" for the disease. In the United States it is commonly called "internal brown spot" (32), occasionally also "internal browning." In South Africa (12) the name "internal brown fleck" is applied to the same disease.

GEOGRAPHIC DISTRIBUTION

The internal brown spot disease of the potato is quite generally distributed wherever potatoes are grown. It has been reported from various places in Holland, Germany, Denmark, Sweden, Norway, Austria, Czechoslovakia, Great Britain, the Dutch East Indies, South Africa, and numerous localities in the United States of America and Canada.

ECONOMIC IMPORTANCE

As a rule internal brown spot is of minor economic importance. Only in certain soils and in certain years does it assume considerable economic importance. In such cases it may practically ruin the crop as far as the consumptive value of the tubers is concerned, though their value as animal food or for starch production is not materially decreased even when severely affected. Cases where the disease decreased the yield of marketable tubers as much as 50 per cent and even more often have been reported (47). During the summer of 1924 the writer observed that more than 50 per cent of the tubers from a potato field in Bennekom, planted with the very susceptible varieties Schotsche Muis and Eigenheimer, were severely affected

and were unfit for human consumption (Fig. 1). Paravicini (35) reports that in some regions in the Dutch East Indies from 80 to 100 per cent of the tubers may become affected.

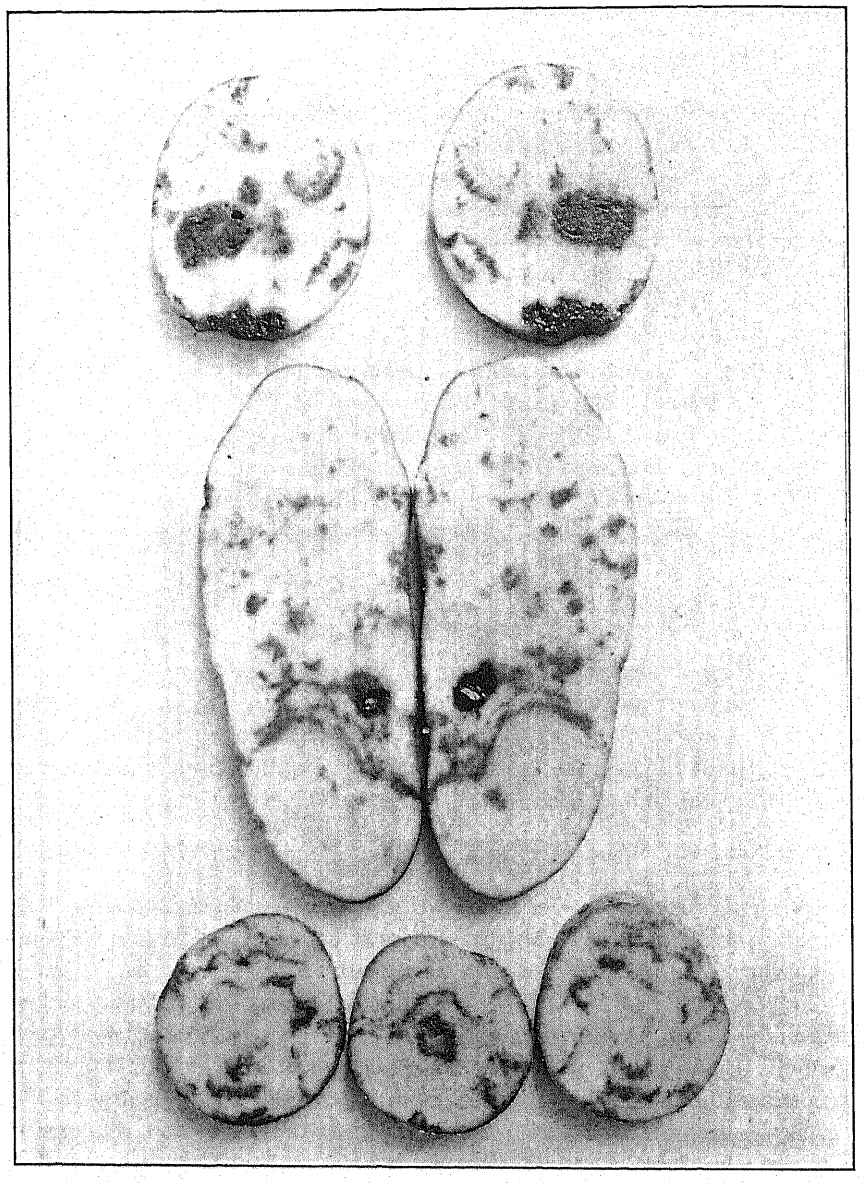

FIG. 1. Cross and longitudinal sections of Schotsche Muis (Midlothian Early) tubers, showing internal brown spot symptoms.

DESCRIPTION OF THE DISEASE

In recent years it has been shown that brown spotting of the flesh of potato tubers is not only characteristic of the internal brown spot disease but may be brought about also by numerous other diseases and conditions, *e.g.*, frost necrosis (24), net necrosis (32), stipple-streak (Atanasoff), etc. Much of what has been considered up to now as internal brown spot has been net necrosis. Therefore, an exact and detailed description of the disease in question seems desirable.

External Symptoms. The disease appears in the tubers long before they are fully grown or lifted, but it is never, or only seldom, seen in the very young tubers.

In most cases the affected tubers show no external symptoms of the disease whatever. Such is the case with tubers which have been attacked by the disease in a more advanced stage of their development or shortly before ceasing to grow, also of tubers on the market and in storage. Such tubers are dirty, somewhat discolored, and often more or less bruised and injured; thus the external symptoms of the disease are obscured. However, when the tubers are dug carefully and rinsed in water, there will be seen on some of them, and especially on those attacked at an early stage of their development, some very distinct changes. This is especially true of the more susceptible varieties and of those with light-colored skin. Tubers that are attacked by the disease while still rapidly growing have an irregular outline (Fig. 2) and show more or less pronounced deformations and depressions. These depressions may be so deep as to give the tuber a very irregular or semicircular shape. The skin over the depressions is much thicker and rough, often cracking in all directions (Plate XXXVI, A), the cracks giving the tuber a scabby appearance. If the skin over the depressions is not too thick and brown colored, there may be seen, as first reported by Pethybridge (37), dark or chocolate brown blotches underneath in the flesh of the tuber (Plate XXXVI, B). In some of the more susceptible early varieties, as Schotsche Muis for instance, these blotches may be very large and extend deep into the tuber. In such cases secondary organisms develop in the dead tissues and give rise to a large brown or black lesion on the tuber (Fig. 1).

Tubers attacked by the disease shortly before or after reaching their full development are quite normal in shape and size. Their skin, however, shows here and there slight irregularities and rough spots, under which are visible, in the case of newly dug tubers, brown blotches, spots, streaks, or ring-like discolorations of the flesh, which seem to be arranged around a central spot, lying usually under a lenticel. These central spots, as well as the centers of the depressions, apparently represent the original point of infection. When the skin is removed carefully, the discolorations in the flesh of the tuber become even more distinct.

Internal macroscopic symptoms. Upon cutting a tuber longitudinally or in cross section there will be seen dark rusty brown blotches or streaks irregularly spread over the cut surface and having no connection with the vascular ring, stem-end or eyes of the tuber nor apparently with the periderm of the tuber, as observed by numerous authors (Fig. 1). In reality this is not so. When the tubers are cut perpendicularly through the externally visible brown areas, or through the depressions (Fig. 2), which apparently represent the original point of infection, it will be seen that the rusty brown discolorations of the flesh radiate from the center of the depressions or externally visible brown blotches underneath the skin. In tubers infected simultaneously at several points, where the rusty brown blotches, streaks, or rings are numerous and all over the tuber, the above condition is much less pronounced. This is also true of tubers in an advanced stage of the disease (Fig. 1).

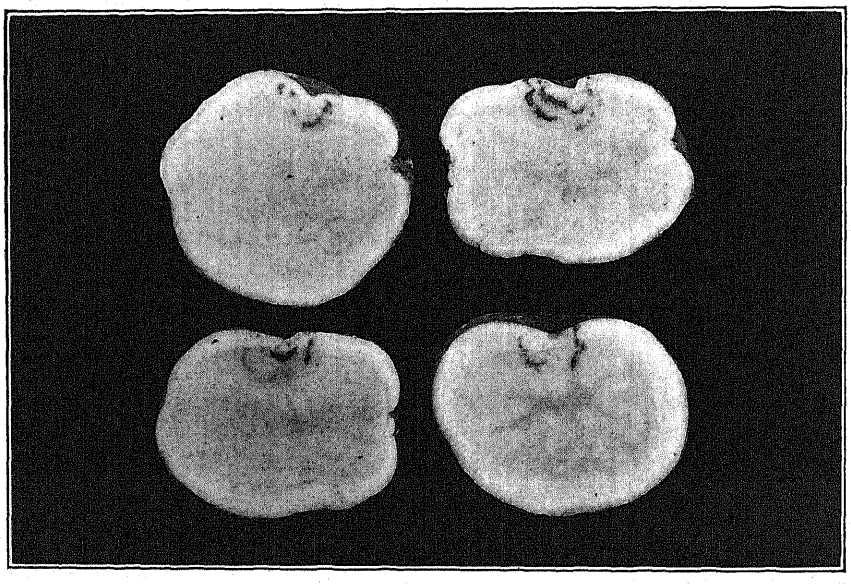

FIG. 2. Longitudinal sections of Eigenheimer tubers showing internal brown spot symptoms. Tubers cut through the depressions, which represent the point of infection. Note the concentric rings and spots around the points of infection.

The form of the brown discolorations may be of two kinds, according to the variety and presumably also to other not fully understood factors. In some varieties they consist mostly or entirely of irregular, rusty brown blotches of varying size and shape. This form of the disease may be mistaken, in superficial examination, for net necrosis. In other varieties, even when growing under exactly the same conditions, the discolorations have

usually or exclusively the shape of streaks, arcs, or rings, always radiating from a certain center (Plate XXXVI). The latter usually lie, in the early stages of the disease, at the surface of the tuber; and it is not unusual to see several brown arcs or more or less complete rings formed concentrically. The rusty brown areas lie more or less sharply outlined in the normal tuber tissues.

The number and extent of the rusty spots and of the browning usually increase considerably during the first months subsequent to the lifting of the potatoes, and remain so during the rest of the life of the tuber. The increasing of the browning is more apparent in the early-lifted tubers than in tubers which have been dug late and in fully ripe condition.

Infected tubers of most varieties, when properly stored, usually will keep as well as healthy tubers. Severely affected tubers, primarily of the more susceptible varieties as Schotsche Muis (Fig. 3) and Eigenheimer, which show deep lesions at the time of lifting, undergo a kind of dry rot during the winter, whereby the whole interior of the tuber becomes brown and perforated by numerous cavities. The dead tissues and cavities are gradually invaded by fungi, mostly Fusaria, and the whole tuber finally becomes a hard mummy.

Internal microscopic symptoms. Hand sections through the brown patches show that they consist of groups of otherwise normal but rusty or reddish brown cells which in most cases are packed with starch grains. These cells are dead. The cell contents, except the starch grains, are shrunken, brown and indistinguishable, while the cell walls seem to be considerably thickened and suberised. No foreign organisms such as fungi, bacteria, etc., are visible in the dead tissues. The brown patches may be of considerable size or may consist of only a few cells. In most cases they are surrounded by a distinct cork layer, consisting of three to six or even more layers of thin cork cells. This is especially true some time after the lifting of the plants. The cork layers, as well as the neighboring cells of the surrounding healthy tissues, are starch-free and translucent, so that a light ring becomes apparent around each blotch when the sections are examined in transmitted light.

ETIOLOGY OF THE DISEASE

The absence of visible pathogenes in the rusty brown tissues of the affected tubers led the students of this disease from the very beginning to consider it to be physiological in nature, presumably caused by disturbed chemical equilibrium in the plant, or by soil or climatic conditions. This is now the most current opinion regarding the nature of internal brown spot.

Former investigations. Numerous workers have searched for the pathogene of this disease, but Swellengrebel in Holland and Paine in England are the only two workers who claim to have succeeded.

Swellengrebel (52), in an extensive paper on this subject, was the first to attribute the cause of the disease to bacteria. By removing portions of the brown tuber tissues under aseptic conditions and placing them on sterile boiled potato plugs, Swellengrebel found that the affected tissues are not sterile, as claimed by former workers, but contain bacteria. In most cases he isolated the following four bacteria: *Bacillus megatherium* De Bary, *Bacillus vulgatus* Migula, *Bacillus mesentericus* L. et N., and *Bacterium punctatum* L. et N. Swellengrebel considers these bacteria not as specific pathogenes, but as harmless saprophytes present everywhere in the soil and on the potato tubers, under ordinary conditions harmless, but evidently

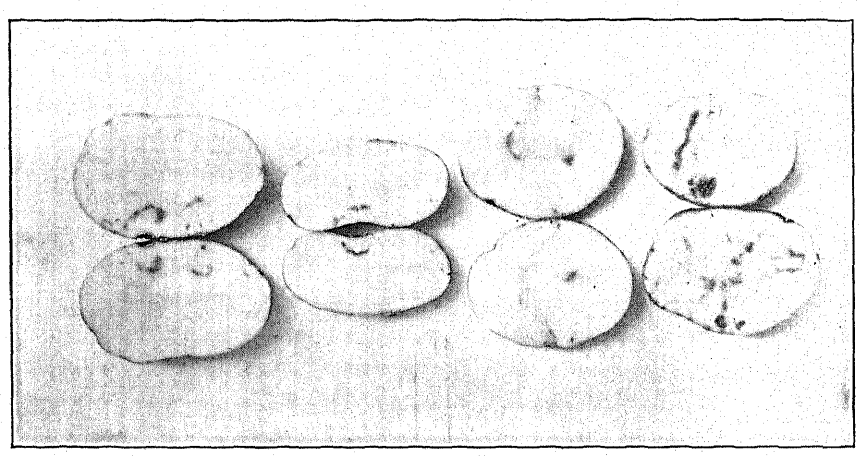

FIG. 3. Schotsche Muis (Midlothian Early) tubers cut longitudinally to show the symptoms of internal brown spot.

becoming parasitic under certain conditions. New tubers inoculated with pure cultures of the four bacteria produced, after several months, in some cases a rusty brown discoloration of the tissues around the wound in which the inoculum was deposited. The brown tissues showed the same microscopic picture as those of tubers naturally infected with internal brown spot. This browning of several cell layers around the point of inoculation, which did not appear in the control tubers, was considered by Swellengrebel as proof that each of the above four organisms independently is able to cause the disease.

Paine, in 1918 (33) and 1923 (34), reported that he had repeatedly isolated a bacterium from the affected tissues of diseased tubers. This organism, which he calls *Pseudomonas solaniolens*, is a small, oval, non-sporing rod, motile by a very long, single polar flagellum, and has a penetrating smell of earthy potato.

Paine infected tubers by inoculating with cultures of this organism inserted into a hole in the tuber, two or three centimeters deep and half a centimeter wide, made with a sterile carpenter's auger. The hole was plugged with a core of potato cut with a sterile cork-borer from another tuber of the same variety. The inoculated tuber was either planted in soil or kept in a waxed cardboard box. "After three or four weeks incubation of material inoculated in this way one finds the core reduced to a hard shrivelled corky mass, the hole usually widened towards the bottom and walls thickly suberised with a brown mass of cork having finger-like projections running out into the surrounding tissue; the suberised walls of the cavity are usually about two to three millimeters thick." The suberization, Paine finds, is exactly like that of the naturally affected tubers. "In two or three experiments threads of cork have been traced running into the tuber for more than a centimeter, and these exactly resemble those of net necrosis. We thus have both types 'A' and 'B' arising from the same inoculation." Paine (34) distinguishes two types of the disease: type 'A' represents the internal brown spot proper, and type 'B' the disease known in the United States and England as "net necrosis."

EXPERIMENTAL METHODS OF ISOLATION

The writer repeated Swellengrebel's and Paine's work. In numerous isolation experiments he found that occasionally the affected tissues of the tubers contain bacteria. So far, however, none of the organisms isolated from diseased tubers have produced the symptoms of the disease observed in nature, although most of them have produced a local browning and suberization of the tuber tissues around the point of infection.

The freshly dug, but externally uninjured, diseased tubers[1] are thoroughly washed in water with soap and brush, then disinfected in 2 per cent $HgCl_2$ for 3 to 4 hours, and subsequently held in a flame and gently rotated so as to burn off the skin of the tuber on all sides. The tuber thus treated, so far held with forceps, is placed in the left hand, which previously has been thoroughly washed and disinfected. The tuber is held at one end and only with the tips of the fingers. The cortex of the tuber is then removed with a flame-sterilized, all-steel scalpel, which has been cooled by dipping in boiling water. From the peeled end of the tuber are cut square blocks, large enough to fit into a culture tube. The potato plugs are then placed in culture tubes containing about 10 c.c. of sterile water and held ready by a second person. A large number of such tubes are prepared each time. The tubes to which large portions of the affected tubers have thus been

[1] New tubers were used throughout the isolation work on account of the possibility that the pathogene might die out in the older tubers. Besides, the probability is much greater that old tubers will subsequently become invaded by saprophytic organisms.

added are incubated for several weeks at 20° C. Owing to the osmosis which takes place under such conditions, a considerable portion of the protoplasm and soluble cell contents of the potato plug passes into the water and makes it a highly suitable culture medium for the growth of bacteria and other organisms. That not only the soluble cell contents but also the cell protoplasm diffuses into the water can easily be shown by warming up such tubes. This causes a coagulation of the protoplasm, and the liquid content of the tubes becomes milky.

If any organisms actually are present in the affected tuber tissues, they most likely will appear in the liquid outside of the living potato plugs and become visible through clouding or other visible changes in the liquid. In fact a considerable number of the four hundred or more test tubes prepared in this way became clouded within two to three weeks owing to the development of bacteria in them. The percentage of clouded tubes varied from time to time from 10 to 30 per cent, while similar test tubes prepared from healthy tubers seldom showed any bacteria, although in both cases each time a small number of tubes became contaminated with *Penicillium*.

Infection experiments with bacterial cultures isolated in the above way from tubers affected with internal brown spot have so far given no conclusive results.

Pathologic changes in potato tubers as produced by Swellengrebel and Paine with the alleged pathogenes of this disease have been repeatedly produced by the writer, not only with cultures of different bacteria isolated from diseased tubers, but also with a large number of other bacteria isolated from soils where the disease never does occur, and from other sources. Often the characteristic rusty discoloration of the tissues around the point of infection was very pronounced and penetrated farther than in Paine's experiments. But in all cases the discoloration, though identical with that produced in naturally infected tubers, remained limited to the tissues surrounding the point of infection and never extended into the interior of the tuber as in the case of naturally infected tubers.

The inability to produce experimentally the symptoms of the disease as observed in nature can not be due to the fact that the environmental conditions in the experimental work, and the conditions of the tuber itself, differ from the conditions under which the disease develops in nature, as Paine (34) is inclined to believe. That this is so is shown by the fact that, when a piece of a naturally infected tuber is grafted to a newly dug or still fresh and healthy tuber, the disease will develop in the healthy tuber in a most typical form within two or three months, and brown blotches and typical rusty brown arcs and rings will appear far away from the graft in the parenchyma of the healthy tuber. This experiment has been done often by the writer, and, as long as the tubers used were new and fresh, each time with the same results.

It may be that some of the organisms isolated from diseased tubers are actually the cause of the disease. It is possible that they may rapidly lose their pathogenicity outside of the tuber or in artificial culture. In all cases the writer has used cultures isolated only from two to four weeks previously from affected tubers, but, as already stated, in all cases the results were negative. Moreover, it is reasonable to expect that organisms which are virulent enough to cause distinct and very typical browning of the tissues around the point of infection, occasionally to a depth of more than one centimeter, should be able, if identical with the cause of the disease, to penetrate farther and bring about the symptoms of the disease seen in naturally infected tubers.

DISCUSSION AND SUMMARY

The experimental evidence to date regarding the cause of the internal brown spot of the potato tuber is in no way convincing.

The following facts, however, show that it is not a physiological disease and throw some light on its nature:

1. Under ordinary conditions in Holland internal brown spot of potatoes occurs constantly in a more or less severe form on certain soils primarily rich in organic matter, or heavily manured, light, sandy loam, or sandy soils, and reclaimed muck lands. It has never been seen in Holland on heavy clay soils, even only a short distance from muck and sandy soils where the disease is very common.

2. The writer's observations during a period of five seasons have convinced him that lack of moisture can not be the cause of the disease, as is claimed by some. On the contrary, sufficient moisture seems to be a prerequisite for the development of this disease in severe form. The only year, out of five, during which the disease caused but little injury in a field where it severely attacked the tubers during the other four years, was the unusually dry summer of 1921. During this season the disease was practically absent. The early portion of the summer of 1925 was also very dry and warm. The early variety Schotsche Muis (Midlothian Early), which is very susceptible to internal brown spot, was perfectly free from it up to June 15, at which time the plants were almost ripe for lifting. Subsequent to June 15 continuous rains came and the air temperature fell. Ten days later numerous cases of internal brown spot occurred.

3. The disease is not transmitted with the potato seed.

4. The causal agent of the disease enters the tubers from without, bringing about distinct pathologic changes at the point of entrance to the tuber. These consist of distinct scabbing of the tuber periderm and slight or pronounced depressions in, and deformations of, the tubers. The subsequent browning of the tuber parenchyma radiates from the point of infection, usually visible on the outside of the tuber.

From the above it could be concluded that the cause of the disease is an organism or a chemical substance. The latter seems less probable, because it is not very likely that at certain points in the soil, especially in wet seasons, the concentration of a given substance can be so much higher than at other points around the tuber as to cause a local and not a general poisoning of the tuber surface.

5. The disease can easily be transmitted to fresh healthy tubers by grafting portions of diseased tubers on them. This also is in favor of the supposition that the disease is caused by a living organism and is not due to a local poisoning of the tuber. Were the latter the case, the poisonous substance which might have entered the first tuber and brought about the death of local areas would at last become so diluted or bound that it would not be able to pass to any extent into the grafted tuber, at least not deep into the interior of the healthy tubers, as was always the case in the writer's graft experiments. Even less probable seems the transmissibility of the effect of given unfavorable environmental conditions from the diseased tuber portions to the healthy tubers grafted with them.

INSTITUUT VOOR PHYTOPATHOLOGIE,
WAGENINGEN, HOLLAND.

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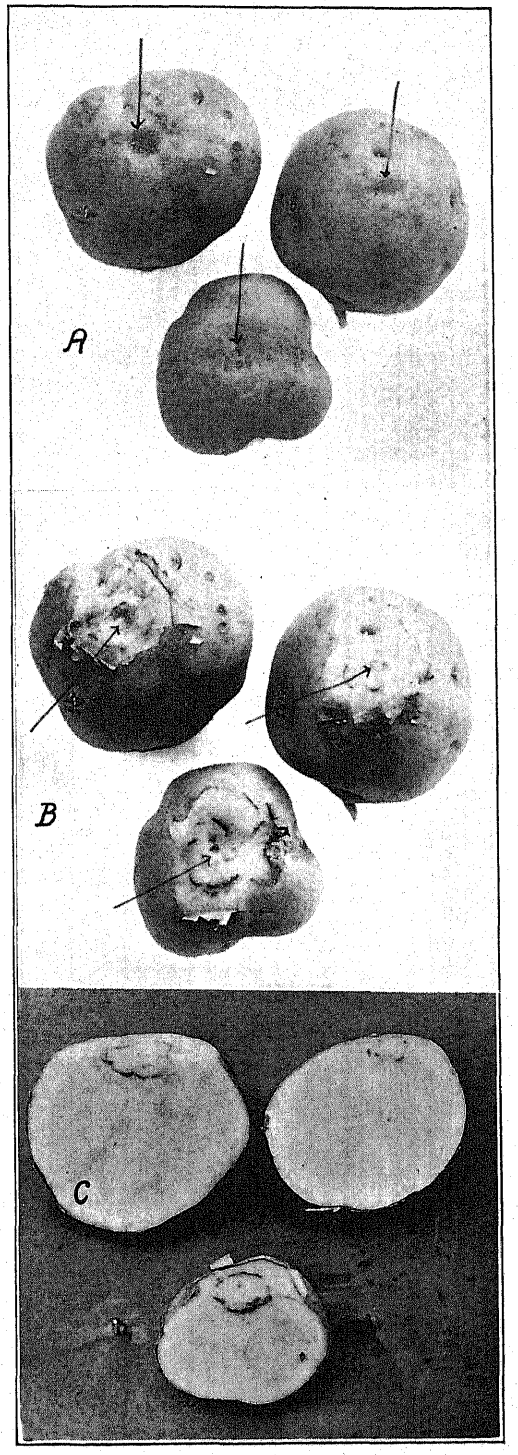
EXPLANATION OF PLATE XXXVI

A. Eigenheimer tubers showing scabbing at and around the point of infection with internal brown spot.

B. Same tubers with scabbed epidermis, removed to expose the points of infection, and the brown spots and rings radiating from them.

C. Same tubers cut through the point of infection. Note the arrangements of brown spots and rings around the points of infection.


A
B
C

THE MORPHOLOGY AND THE PATHOGENICITY OF SOME PHYTOPHTHORA MUTATIONS[1]

LEON H. LEONIAN

Although physiology forms the chief basis of bacteriological classification, mycologists still cling to the purely morphological methods. As a result of this conservative policy many groups of fungi are extremely confusing not only to the average investigator but even to the so-called specialist.

It is the purpose of this paper to demonstrate how easily one may, if strict morphological methods are followed, make new species almost at will, and how infinitely difficult it is to identify these species after they are once made.

The mutations of the rhubarb *Phytophthora* (formerly *P. parasitica* var. *rhei,* but more recently included[2] in *P. omnivora*) have been used for the purpose outlined in the foregoing paragraph. Plate XXXVII illustrates the colony characteristics of these five types. All of these organisms came from a single sporangium and breed true with the exception of types I and IV. These latter two organisms represent what may be conveniently termed reversible mutations: one type may revert to the other or it may give rise to a complex colony consisting of both types. This phenomenon has been described in a former paper.[2]

The five colonies illustrated in Plate XXXVII arose as mutations from the rhubarb *Phytophthora.* They are so unlike each other that no one would take them to be members of the same species and grown under identical conditions. It is probable that similar cases have been responsible for the description of many new species which after a careful investigation have failed to show a good specific value.

There are further differences in these five types. Figure 1 shows the character of the submerged mycelium and it can be seen that types I, II, and III have very irregular, gnarled, profusely branching, and bulbous hyphae, whereas those of types IV and V are fine and even.

Types I and II produce oogonia in abundance, type V not so readily, type IV sparingly, and type III none at all. Yet the presence or the abundance of oogonia are considered by the morphologists to be of very important taxonomic value.

[1] Published with the approval of the Director of West Virginia Experiment Station as scientific paper No. 21.

[2] Leonian, Leon H. Physiological studies on the genus *Phytophthora.* Amer. Jour. Bot. 12: 444–498. 1925.

Perhaps the most important features used by the morphologists in the classification of Phytophthoras are the size and the shape of sporangia. Whenever an investigator finds a Phytophthora strain which has sporangia differing a little from those of the described species, he is apt to think that mycology is about to receive another important addition to the list of new species. The micron, however, is not as efficient as it appears to most mycologists, as can be seen from the following experiment: Rhubarb plants

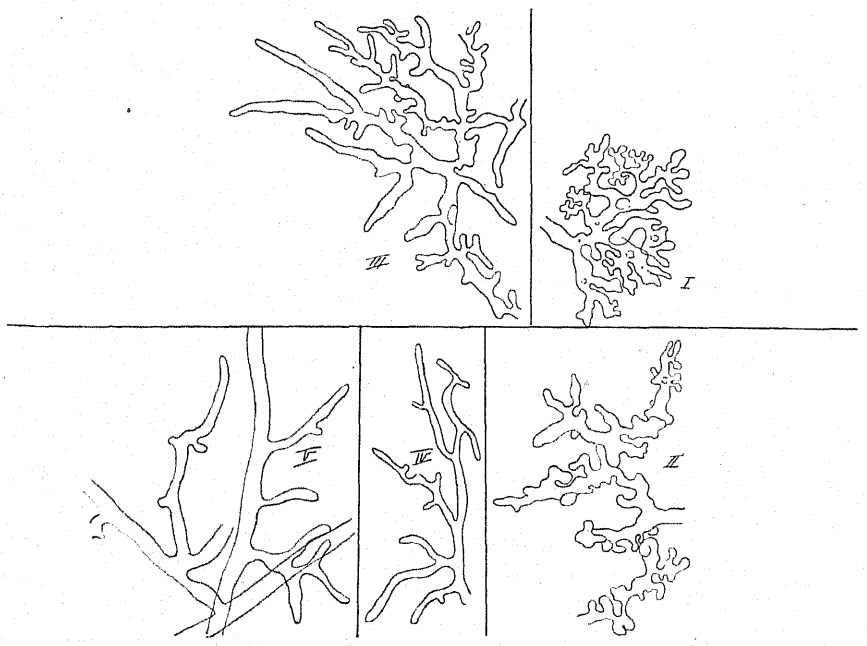


FIG. 1.—Submerged hyphae of the five types of rhubarb *Phytophthora.*

were inoculated with the five types of *Phytophthora* mentioned above. After the infection occurred, two hundred sporangia were measured from each of the five series. Since no sporangia are produced on the host under natural conditions, and since types III and V attack the host with great difficulty if at all, water cultures were tried and found satisfactory. Young rhubarb plants were transferred to Pfeffer's solution and inoculated. The moist condition induced the formation of profuse aerial hyphae upon the infected tissues and within two days large numbers of mature sporangia appeared. Several plants were used to furnish the sporangia of each type, and inoculated plants from two or more series were included in order to make the measurements more representative. Table 1 gives the summary of these measurements.

TABLE 1.—*Frequency distributions in percentages and constants in microns for length and width of 200 sporangia of five types of Phytophthora*

Class centers in microns	type I		type II		type III		type IV		type V	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
7.0	0	0	0	0	0	0	3	3	0	0
10.5	0	0	0	0	0	0	8	12	0	0
14.0	0	1	0	0	0	0	18	23	1	1
17.5	2	1	0	0	0	0	34	34	1	4
21.0	1	4	0	1	0	0	15	11	4	8
24.5	1	4	1	3	1	5	11	10	10	14
28.0	3	6	1	9	2	5	6	5	15	20
31.5	7	13	3	14	3	15	1	1	15	22
35.0	13	24	15	30	10	25	1	1	18	17
38.5	8	15	7	17	8	17	1	0	11	8
42.0	8	21	21	15	18	24	2	0	10	5
45.5	15	7	13	8	16	5	0	0	6	0
49.0	4	3	12	3	16	2	0	0	5	0
52.5	14	1	15	1	17	1	0	0	3	0
56.0	6	0	7	0	4	0	0	0	1	0
59.5	8	0	4	0	4	0	0	0	0	0
63.0	4	0	1	0	1	0	0	0	0	0
66.5	3	0	0	0	0	0	0	0	0	0
70.0	3	0	0	0	0	0	0	0	0	0
Mean size in microns	43.70	33.20	43.70	36.70	43.70	37.30	25.50	21.00	35.00	28.00
Median size in microns	45.56	35.31	42.81	35.35	47.91	35.53	17.92	17.59	34.88	30.06
Mode in microns	45.50	35.00	42.00	35.00	42.00	35.00	17.50	17.50	35.00	31.50

It can be seen that types I, II, and III manifest only small differences in the sizes of their sporangia. But note the sharp drop in spore size of type IV. Less evident differences have served as the basis of establishing many new species of fungi. In this case, however, no such practice can be tolerated because type IV is not fixed in its physiological or morphological habits and may revert at any time to type I. When such reversion occurs the mode for length of sporangia changes from about 18μ to 45.5μ. This reversion is continuous and independent of outside conditions. How can the morphological system classify such a fungus? Shall we say that this

same organism is one species at one time and another species at another time?

The sporangia of type V are also very small and more spherical as compared with the first three. On this basis alone it would undoubtedly be considered as a new species or else a species entirely different from *P. omnivora*.

It has been demonstrated previously that the size and shape of sporangia show wide variations under different environmental conditions; therefore it is only natural to expect that a change of host would induce certain clear-cut differences. In order to test this possibility some green pepper fruits were inoculated in the laboratory with the pure culture of type I of the rhubarb *Phytophthora*. Pepper was found to be a very good host and induced a profuse growth of the fungous mycelium; only a few sporangia formed, however, even after a long period of growth. But when a piece of the infected pepper with the attached mycelium was transferred to a covered glass dish containing a shallow layer of Pfeffer's solution, sporangia formed in great abundance within two days. The sizes of 400 of these sporangia taken from two lots of peppers are shown in the last four series of measurements given in table 2. The two lots of peppers were inoculated at different times. The environmental conditions in all of these experiments have been nearly the same and the temperature was held at approximately 23° C.

A difference of ten to twenty-one microns between the mode lengths, and seven to twelve microns in the mode width of sporangia of the same type of fungus when grown on rhubarb and pepper respectively, must seem rather disconcerting to those who have not hesitated to accept a margin of from three to five microns as sufficient in specific differentiations. Yet it readily can be seen that in the foregoing four measurements there is a fluctuating margin of nine microns in the lengths and about five microns in the widths.

It has been quite a vogue among some mycologists to classify the different types and sizes of spores on the percentage basis and to make it an important factor in identification work. The foregoing tables, however, do not encourage such a practice.

The mycelium which grew from infected peppers was used to inoculate another series of rhubarb plants. After this host was killed and the sporangia appeared, a number of measurements were made. It can be seen from the first two series of measurements in table 2 that the size of sporangia from the rhubarb host was noticeably increased ($49 \times 42\ \mu$ as against $45.5 \times 35\ \mu$) when the fungus was cultured on the pepper and the in-

TABLE 2.—*Frequency distributions in percentages and constants in microns for length and width of 400 sporangia of Phytophthora type I grown on rhubarb and on pepper*

Class centers in microns	On rhubarb		On rhubarb from pepper		On pepper Lot 1		On pepper Lot 2		On pepper Lot 3		On pepper Lot 4	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
7	0	0	0	0	0	0	0	0	0	0	0	0
10.5	0	0	0	0	0	0	0	0	0	0	0	0
14	0	1	0	0	0	2	0	0	0	5	0	0
17.5	2	1	0	1	5	9	3	9	10	14	6	10
21	1	4	0	4	11	21	11	14	10	16	13	23
24.5	1	4	2	8	13	15	15	21	12	19	17	23
28	3	6	5	3	16	26	12	15	14	15	13	13
31.5	7	13	2	4	15	14	12	21	12	14	9	10
35	13	24	3	15	13	10	16	11	12	10	13	12
38.5	8	15	4	15	11	2	12	8	9	4	10	6
42	8	21	7	30	9	1	11	1	8	2	8	1
45.5	15	7	7	10	2	0	3	0	5	1	2	2
49	4	3	23	5	3	0	2	0	3	0	4	0
52.5	14	1	20	5	2	0	3	0	3	0	3	0
56	6	0	14	0	0	0	0	0	0	0	1	0
59.5	8	0	8	0	0	0	0	0	0	0	1	0
63	4	0	5	0	0	0	0	0	0	0	0	0
66.5	3	0	0	0	0	0	0	0	0	0	0	0
70	3	0	0	0	0	0	0	0	0	0	0	0
Mean size in microns	43.70	33.20	43.75	35.00	35.00	28.00	35.00	29.70	37.30	29.70	38.40	31.40
Median size in microns	45.56	35.31	41.00	49.00	31.43	27.37	34.26	30.45	29.13	24.96	34.36	25.05
Mode in microns	45.50	35.00	49.00	42.00	28.00	28.00	35.00	24.50	28.00	24.50	24.50	22.75

oculated into rhubarb. This increase, however, is not considered as a carrying-over effect, but merely a normal fluctuation.

Neither can the shape of sporangia be considered a significant point in taxonomy. A glance at figure 2 dispels any illusion that one may have con-

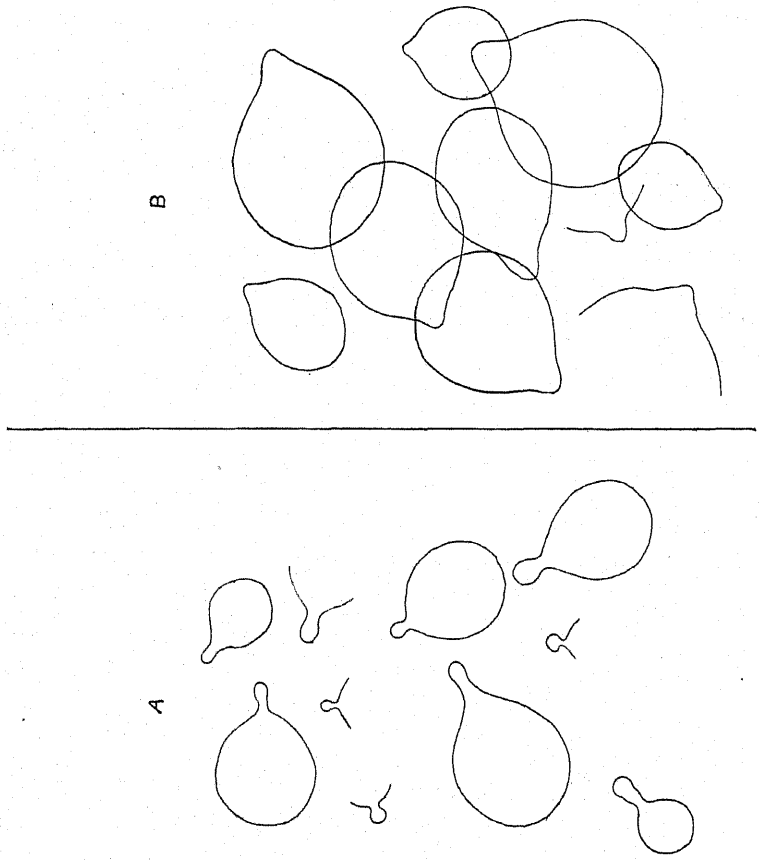


FIG. 2.—The relative sizes and shapes of sporangia of the rhubarb *Phytophthora* type I when grown on pepper fruit, A, and on rhubarb, B.

cerning the value of the shape of sporangia. In addition to the small size the sporangia from the pepper fruit usually possess very prominent papillae as contrasted with the much less pronounced papillae of the sporangia from rhubarb plants.

Morphologists have also considered the pathogenicity of an organism as an important and dependable character, but experiments conducted with the rhubarb *Phytophthora* and its mutations indicate that host relationships are no more reliable or significant, at least in this organism, than spore size.

Hundreds of rhubarb plants, young and old, were inoculated with the foregoing five types. All possible precautions were taken to eliminate errors. Disinfected seeds were grown in sterilized soil and the seedlings were later transplanted to steamed soil in the greenhouse and inoculated.

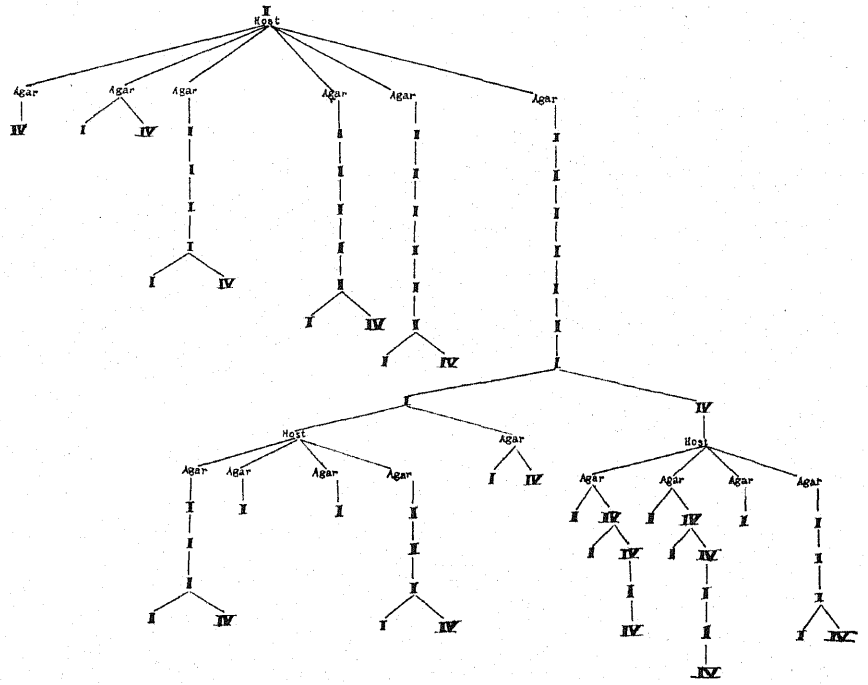


FIG. 3.—Chart illustrating the behavior of the rhubarb *Phytophthora* type I. The host was inoculated with a pure culture of type I; after the death of the plant six isolations were made on agar. Note the reversion, the splitting into two forms, the breeding true for a number of generations and then splitting and reverting again, thus illustrating a most remarkable plasticity and reversible mutations.

Numerous isolations were made from the infected plants to be certain that no other organism was a causal factor. These inoculation experiments were extended over nearly a year. A large number of full grown plants were used in addition to the seedlings and younger plants, and it has been concluded that types I, II, and IV are strongly pathogenic while types III and V can be eliminated as of no economic importance. Only very rarely did type III kill the host outright. Whenever an infection occurred it was confined to only one or two leaves and soon disappeared leaving no bad effect upon the host. Type V was unable to kill the host and only rarely was it able to attack a leaf or two. Even in water cultures where the host was weakened and the mycelium was stuffed inside of the host tissues, great

difficulty was experienced in inducing satisfactory infection. Christensen[3] has observed similar physiological specialization in the mutations of *Helminthosporium sativum*.

It has been seen that the nature of fungous colonies, the shape of the submerged hyphae, the presence or the absence of oogonia, the shape and the size of sporangia, and finally the pathogenicity of these organisms are not dependable factors, and if one is to follow such characters exclusively, confusing and even ridiculous situations may be expected to arise.

Studies conducted thus far and partially reported[4] indicate that a physiological scheme of classification where a great number of reactions are available can avoid such confusions in taxonomy because apparent specific differences disappear to such an extent that all of the foregoing five types, as well as a number of other supposedly distinct and authentic species and undescribed forms can be classified under one species group, namely *P. omnivora*.

It has been stated before that types I and IV, although appearing as very distinct forms, revert to each other or give rise to complex colonies. All attempts to control this by the manipulation of environmental factors have given negative results. It was hoped that perhaps the host would exert a stabilizing influence and fix the type, but figure 3, which shows the result of this attempt, dispels all such hope.

Reversible mutations are not uncommon; the writer has observed and isolated a great number of these not only in Phytophthoras but also in Fusaria. These phenomena play havoc with the present scheme of classification and they can not be ignored by ascribing them to environmental influences. Until some investigator is more successful in controlling such phenomena they will remain as taxonomic puzzles to be solved only by physiological methods.

SUMMARY

Work with some mutations of *Phytophthora omnivora* DeBary has indicated that the nature of fungous colonies in pure cultures, the shape of the hyphae, the presence or the absence of oogonia, the shape and the size of sporangia, and finally the ability to produce disease on a given host are not dependable factors in the taxonomy of the genus *Phytophthora*.

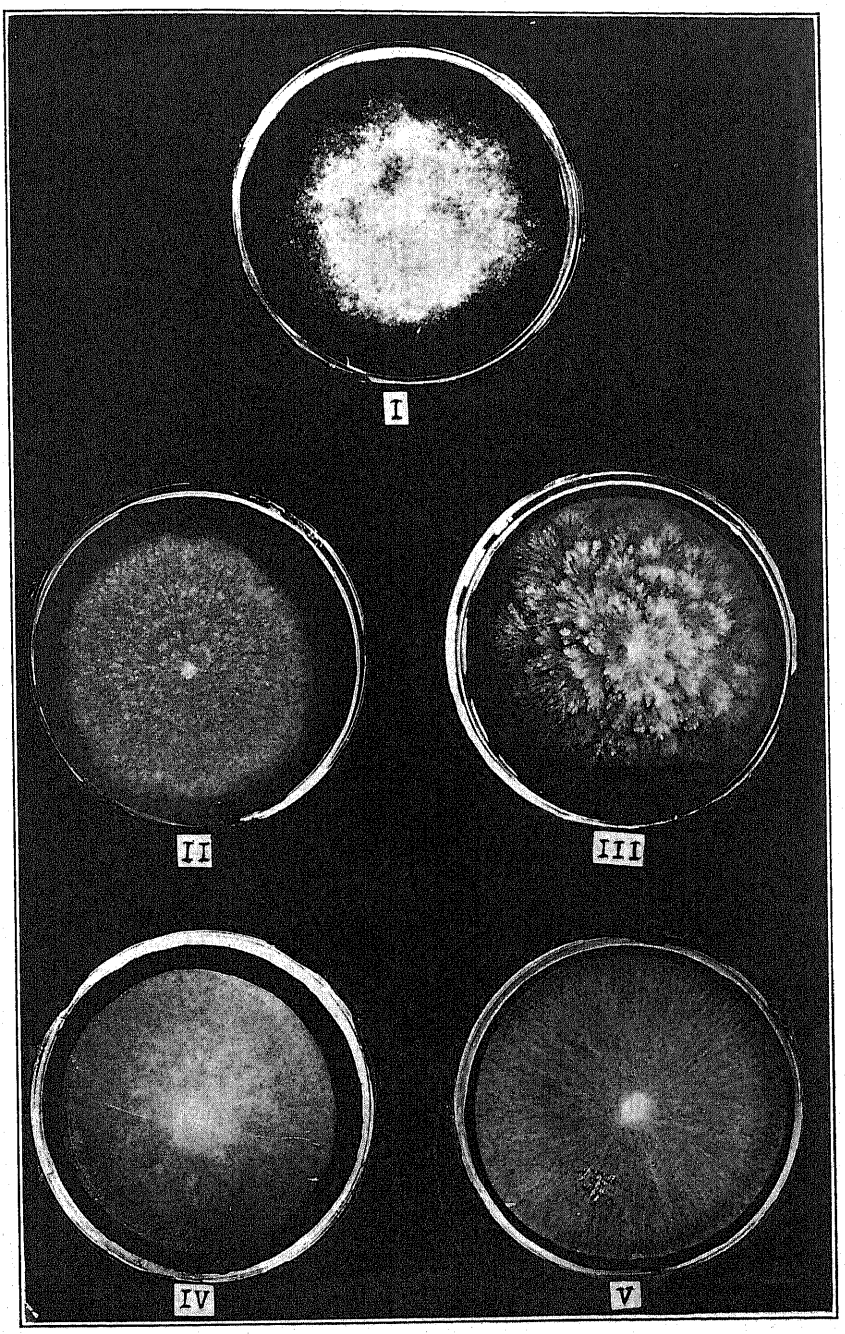
WEST VIRGINIA UNIVERSITY
MORGANTOWN, W. VA.

EXPLANATION OF PLATE XXXVII

Colony characters of the five types of the rhubarb *Phytophthora*. Type I is the original form which gave rise to the other four; it was grown from a single sporangium.

[3] Christensen, J. J. Physiologic specialization and mutation in *Helminthosporium sativum*. Phytopath. 12: 785–795. 1925.

[4] Leonian, *loc. cit.*


I
II
III
IV
V

HEREDITARY ABNORMALITIES RESEMBLING CERTAIN INFECTIOUS DISEASES IN BEANS

WALTER H. BURKHOLDER AND ALBERT S. MULLER

Two striking abnormalities of the bean plant, *Phaseolus vulgaris* L., have come under the observation of the writers during the past few years, each so similar to a common disease of this crop that some confusion was experienced before it was learned to distinguish them from the diseases they resembled. That they could not be classified as diseases was determined when it was found that fungi, bacteria, or infectious principles were not associated with them, but that they appeared to be transmitted only to the progeny of affected plants, and then as recessive characters. Genetical literature is full of cases of abnormalities in plants being inherited, but in very few instances have these abnormalities been mistaken for diseases. Such a one, however, is reported by Dr. R. A. Emerson[1] in the case of blotch leaf in maize. The appearance of the blotch on this plant suggests a fungous or a bacterial disease, but the character is inherited as a recessive, as he has shown. The two abnormalities found in beans and discussed in this article are: first, a leaf character similar in appearance to the symptoms on leaves caused by the mosaic disease; and, second, a seedling wilt, for a time thought to be due to a fungous or bacterial infection.

THE PSEUDO-MOSAIC

The term *pseudo-mosaic* was applied to this character of the bean leaf for convenience, to distinguish it from the true mosaic of this plant. In appearance the pseudo-mosaic differs from the true disease in the following characters. The leaves are narrower, longer, and have more or less of a tendency to twist. There is also a more striking difference between the lighter and darker green areas. The dark areas are fairly normal in appearance, and not so raised or cupped as in the true mosaic. The light areas, on the other hand, are frequently yellowish, and occur at times as a series of streaks on the leaves. If examined closely, one can observe a fasciation or an anastomosing of the veins as the nucleus of the lighter areas. The increase of the vascular tissue is, no doubt, the main cause of the yellow appearance in these areas, but there also appears to be less chlorophyll in the surrounding cells.

[1] Emerson, R. A. The inheritance of blotch leaf in maize. N. Y. (Cornell) Agr. Exp. Sta. Mem. 70: 1–16. 1923.

The true mosaic frequently changes the type of growth of the bean plant and gives it a bushy appearance with spindly runners. On the other hand, the effect of pseudo-mosaic is limited to the leaves entirely. The yield is not affected by this abnormality, but this is no criterion for dis-

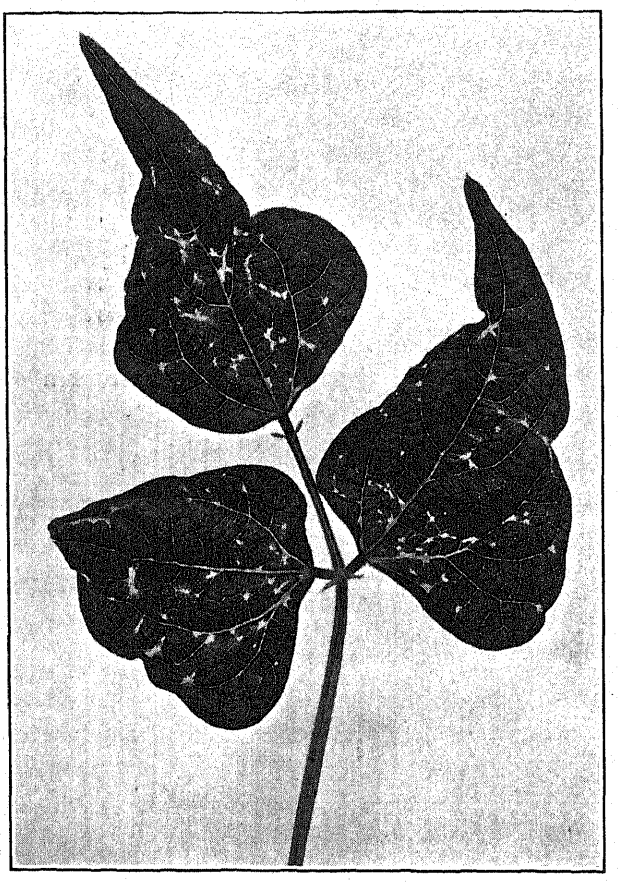


FIG. 1. The pseudomosaic: an hereditary abnormality of bean leaves. The photograph was taken with a ray filter which caused the anastomosed veins to stand out very clearly.

tinguishing the two, since certain varieties of beans will produce a good yield when practically all their leaves show unmistakable symptoms of the true mosaic.

A chart obtained by the Department of Plant Pathology at Cornell University from the U. S. Bureau of Plant Industry, labelled chart 8 Bean Mosaic, CT. 3304, gives in colors very distinctly these two types of leaf

characters, of the true mosaic, and of the pseudo-mosaic. In examining these charts, one may notice that the two top leaves show symptoms of the mosaic disease. The two lower leaves in appearance are typical examples of the pseudo-mosaic dealt with in this paper. Whether the originals of these two lower pictures were a variation of the true mosaic, or a different mosaic, or pseudo-mosaic, it is impossible to state.

The pseudo-mosaic was first brought to our attention in the summer of 1919, when one selection of beans (3292) grown on the experimental plots at Perry, New York, showed 100 per cent pseudo-mosaic. This selection had come from a cross between a White Marrow and a Flat Marrow, and was being grown for its resistance to the root rot caused by *Fusarium martii* f. sp. *phaseoli* Burkh. At that time, while we had noted the two types of mosaic in the bean fields of New York State, we were inclined to consider them variations of the same disease. However, since the type of mosaic on this selection was the uncommon type and there was 100 per cent infection, it was looked upon with some misgivings. None of our selections had ever shown an approach to 100 per cent through natural infection.

Consequently, some inoculation experiments were conducted with this type of mosaic which we now term pseudo-mosaic. At that time on the experimental plots we had growing approximately 100 varieties of beans. Ten plants of each variety were selected and inoculated by rubbing an abnormal leaf over a leaf on one of the healthy plants, care being taken that the epidermal tissues of the healthy leaf were broken in the process. While a small percentage of mosaic developed on the inoculated plants, in no case was the percentage higher than on the remainder of the uninoculated plants. The mosaic which did develop was of the common type and was probably brought in by insects. The indication from these inoculations was that the pseudo-mosaic was not infectious, and further experiments in the greenhouse seemed to corroborate this. It was also noticed that this new type of mosaic had no effect upon the yield. Selection 3292 bore heavily that season; the seed was saved and planted in the greenhouse the following winter and in the field during the summer of 1920. All seeds produced plants having this peculiar leaf character. Seeds from a bean plant affected with the true mosaic seldom give rise to 50 per cent diseased plants. It should be stated here that the pseudo-mosaic character in the greenhouse is not very distinct at times. In the gardens, too, it may vary considerably in intensity of character from year to year. Late in the season the intensity of the abnormality is greater as a rule, and has more the appearance of the mosaic disease. From this it would appear that the character is affected by the environment, a fact which is also true of the symptoms of common mosaic on the leaves.

This problem was not followed up immediately, but in the winter of 1923 a number of crosses were made between selection 3292 and the Robust

Pea bean. Reciprocal crosses were made. In all, 19 pods bearing 53 seeds were secured from these crosses. These seeds were planted during the summer of 1923 and harvested the first of October. Forty-three of the F_1 plants survived and bore seed, but in no case did the pseudo-mosaic character appear on any of the leaves, a fact which indicated that the character, if inherited, was recessive.

During the summer of 1924 the seeds from these F_1 generation hybrids were planted at Ithaca, New York, in a garden removed from all other bean plants; and data were taken on the F_2 individuals. Counts were taken on 34 families containing 1280 individuals. Of these, 75 showed the abnormal leaf character, and 1205 appeared normal. This is quite a close approximation to a 15:1 ratio (1200–80). In no case was any true mosaic observed to complicate the taking of the above observations. Sufficient data seem to have been presented to show that the pseudo-mosaic is not an infectious disease but an abnormality, and one which is hereditary. Good genetical data as to the manner of its inheritance, however, are lacking. To check up the 15:1 ratio obtained in the F_2 generation, it would have been desirable to grow the F_3 plants. This was impossible, however, since the F_2's were late in maturing and were killed by frosts. A further objection might arise as to the use of the Robust Pea bean for one of the parents, when it was known that this bean is resistant to the true mosaic. However, by the use of this variety the chances of a true mosaic entering the experiment are lessened. And, furthermore, McRostie[2] has shown that susceptibility to the mosaic disease is partially dominant, a ratio of 9:7 being obtained in the second generation. Artificial inoculations were necessary in his experiments.

In conclusion we wish to state that, while we hold the pseudo-mosaic to be only an abnormality, similar symptoms on the leaves of beans might be due to a type of mosaic not frequently encountered in the bean fields of New York State.

THE SEEDLING WILT

This abnormality was first brought to our attention by Dr. G. P. McRostie of the Department of Plant Breeding at Cornell University. Dr. McRostie observed, while working on the genetics of the resistance in beans to *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. et C., f. sp. *alpha* Barrus, that a number of his F_2 seedlings died. The crosses were between the Well's Red Kidney and the Robust Pea bean. The trouble outwardly appeared to be an infectious disease of some kind. A great many of the dying seedlings were examined and, although no associated organism was

[2] McRostie, G. P. Inheritance of disease resistance in the common bean. Jour. Amer. Soc. Agron. 13: 15–32. 1921.

found, the writers were inclined to consider it a disease. In appearance and in progress of the trouble the abnormality was as follows. There was no retardation in the germination of the seeds and, unless carefully examined, what appeared to be a healthy seedling would be sent forth. In many of the abnormal seedlings the green was slightly off color. This was

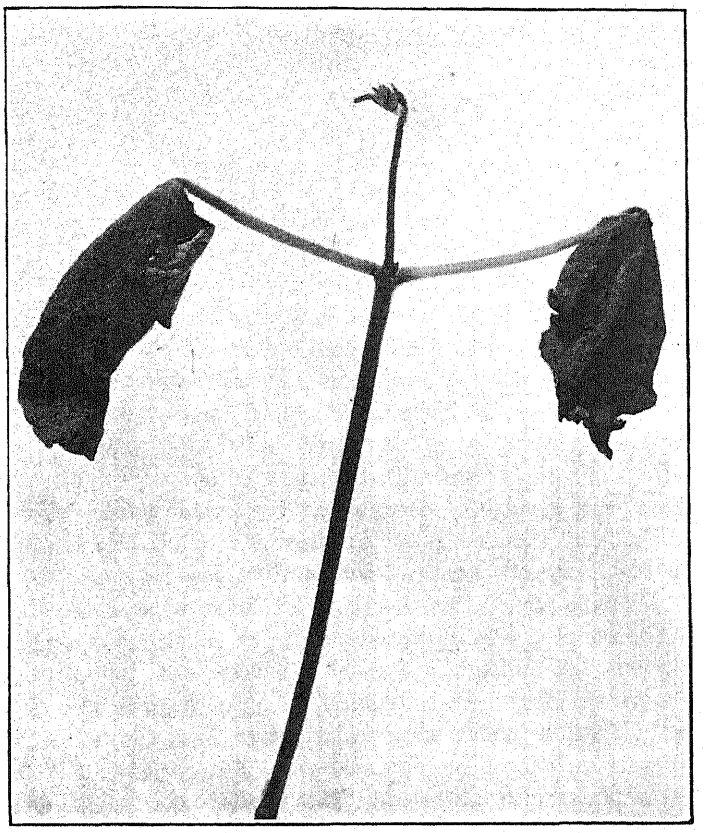


FIG. 2. Hereditary seedling wilt of beans.

hardly noticeable, however, except in contrast with healthy plants. From this stage on, the plants sickened and died. There was no rapid flagging of the leaves as is frequently the case with certain bacterial infections, but otherwise the symptoms were not unlike those produced by certain organisms. The primary leaves became pale and at times had a bronze appearance. They curled somewhat and frequently dropped off. Further growth was seldom made, although, in a few cases, where care was taken of the plants, a few more leaves were added and occasionally a flowerbud was formed before the plant finally died.

In examining wilted individuals, no lesions were found upon the plants; the roots were especially clean. The vascular system too, appeared normal, and search for bacteria or fungi was of no avail. A great many plantings of stems, roots and leaves from these affected plants were made in agar in petri dishes, but no pathogene was isolated. Seeds planted in pots which had had wilted seedlings in them produced healthy plants, although the former wilted seedlings were cut up and placed in the soil. From such experiments it became evident that the trouble was not at all, or at least not readily, communicable.

A clearer understanding of the matter was arrived at when it was observed that the seedling wilt only occurred in bean hybrids, including and after the F_2 generation. Furthermore, it did not occur in all hybrids. It was very frequent in crosses between the Well's Red Kidney bean and the Robust Pea bean, but it never was found in crosses between the Well's Red Kidneys and the White Marrow.

During the season of 1922 a great many individuals of the second generation of hybrids were grown, involving crosses among ten different varieties of beans. The work was being done in connection with some breeding work for resistance to the bacterial blight of beans. Among these seedlings a great many wilted plants appeared, so it was considered worth while to determine the ratios of the wilted to the healthy plants and what varieties of beans were involved. Crosses had been made among the following varieties: Baldwin Wonder Wax, California Wonder Pea, Giant Stringless Green Pod, Keeney's Rustless Golden Wax, London Horticultural, Robust Pea, Refugee Green Pod, Scotia, Flat Marrow, and the Well's Red Kidney. The seedling wilt appeared only in those hybrids of which one of the parents was the California Wonder Pea bean, the Robust Pea bean or the London Horticultural bean, but did not always occur under these circumstances. Families which were entirely free from the wilting seedlings appeared, on the whole, to be composed of healthier individuals than those in which this abnormality did occur. This was also observed in the F_3 generation, but no careful measurements were taken on this point. In the F_2 generation of 16 families containing 1795 individuals, 119 wilting seedlings were found, and 1675 normal ones. A 15:1 ratio which this approximates would be 112 to 1683. It would appear from this that two factors were involved, and, when both were absent from the same plant, that individual would be unable to grow beyond the seedling stage. Data taken on the F_3 generation, however, did not fully confirm this.

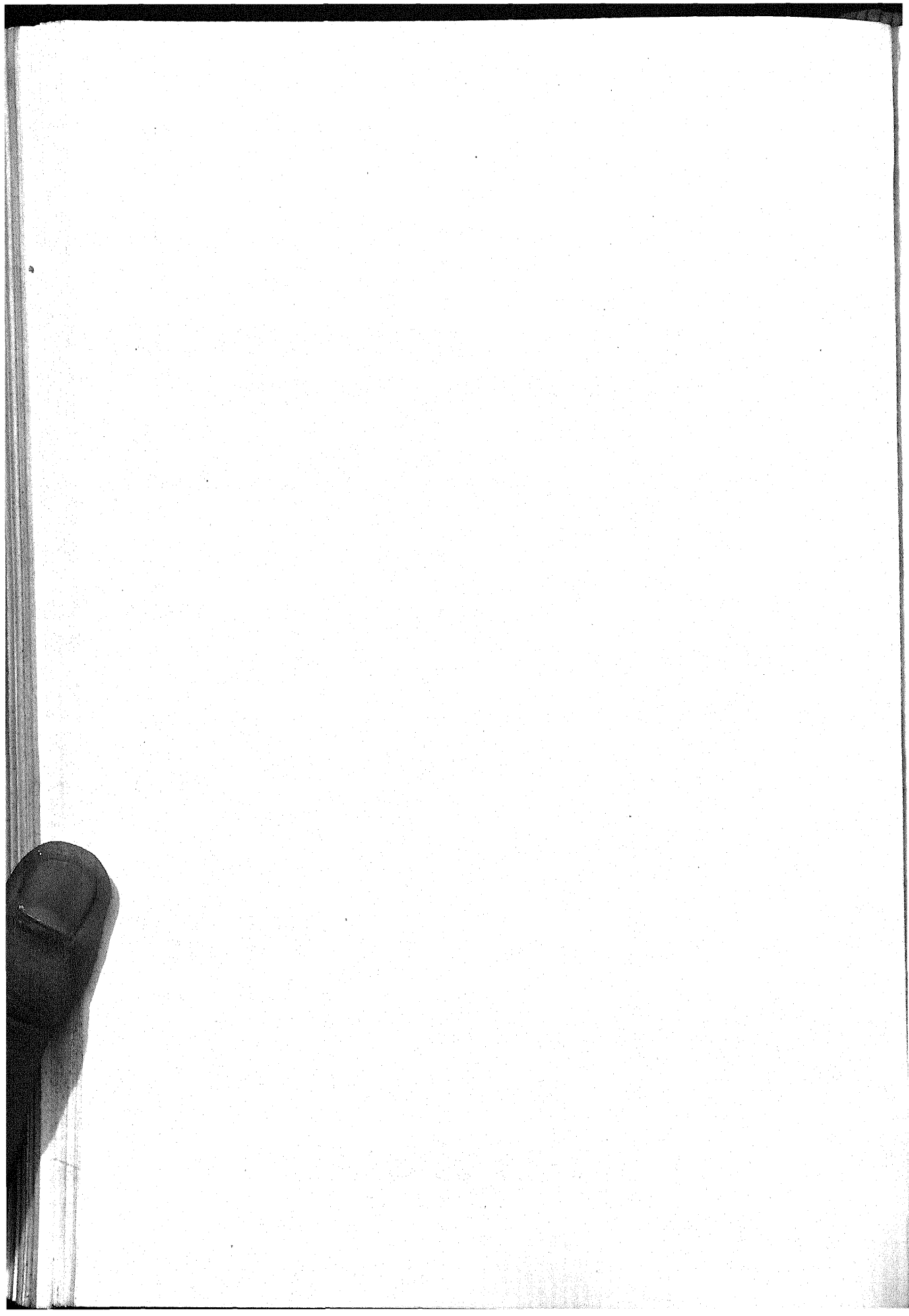
The seeds which produced the F_3 generation were two years old before planting, and the number which was planted was not recorded. Consequently the ratios obtained were based only on the seedlings, and the ungerminated seeds were not considered. These might have changed the

ratios. As it was, over one-half of the families threw no wilting seedlings, and those that did again gave a ratio of 15:1. The 3:1 ratios which were expected did not appear. The above data are by no means complete, especially from the genetical aspect, although we feel that they do show the trouble to be of an hereditary nature.

SUMMARY

Descriptions are given of two abnormalities of the bean plant, *Phaseolus vulgaris* L.: one a leaf character resembling a mosaic, and the other a seedling wilt. Failure to find a causal organism or an infectious principle led to a genetical study of the two characters. Data concerning their mode of inheritance indicate that both are recessive characters and that two factors are involved in each case.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.



THE TEACHING OF ELEMENTARY PLANT PATHOLOGY[1]

L. R. HESLER

The interest which is being manifested in the teaching of plant pathology is encouraging. But no matter where a quickened enthusiasm may lead, we must focus our attention rather steadily upon three vital aspects: the subject, the teacher, and those who are taught. Fundamentally plant pathology is the same wherever and however it is presented. The method used by the teacher is important, and undoubtedly varies widely in its details. But, whatever the method, it is more pertinent to inquire into the philosophy and broad aims of the teacher than to become lost in mere method. The interest of the one who teaches should be in the student as well as in the subject; the teacher should be concerned not so much in whether the student will make a living but how he will think and live.

The course in elementary plant pathology at the University of Tennessee is organized on the crop-basis. It is believed that this plan is at present well suited to the needs of the students taking the course, although the plan, both in general and in detail, is ever subject to new developments in plant pathology and in other fields of science. The general trend of education, it is hoped, will also exert an influence on the form of the course here considered. It is always wise that the teacher shall seek new points of view to which he may shift in order to lessen the danger that the landscape may become worn out by familiarity.

All students registered for the Bachelor of Science in Agriculture degree are required to take the course in the second semester of the sophomore year. (Recent action of the faculty places the course in the fall term of the junior year.) Moreover, all students in the two-year agricultural course are held for plant pathology. Further, the course is designated as an elective for liberal arts students, although with few exceptions only agricultural students take it.

Prerequisite botany consists of a four-hour semester course in fundamental plant studies, dealing largely with elementary plant physiology, with sufficient anatomy included to assist in an understanding of functions. Agricultural students are required to take this course in the freshman year. In the first half of the sophomore year, four semester-hours of general bacteriology are required. The elements of physiological and structural botany and bacteriology are, then, courses prerequisite to our first

[1] Invitation paper read before the American Phytopathological Society, Kansas City, December, 1925.

course in plant pathology. At the beginning of the first term of the junior year these students begin the study of plant diseases.

The course at Tennessee is a four-hour subject. The meetings consist of two lectures of one hour each and two laboratory periods of two hours each. Although most of the subjects in the agricultural curriculum are three hour courses, the curriculum committee has made elementary plant pathology, along with the more general sciences, a four-hour course.

At the outset, let us not overemphasize the importance of the method of classifying the subject-matter. There are perhaps several good plans on which a course in elementary plant pathology might be built, but undoubtedly much more depends on the teacher than on the plan of organization. It is contended, however, that some well-laid plan is necessary. But it should not be forgotten that the student may fail to appreciate the elegance of the plan; what he sees, after all, is not the plan but the teacher.

The crop-basis plan of presentation characterizes the course in elementary plant pathology at the University of Tennessee. With minor exceptions, the diseases of a given crop are studied in sequence, although this does not mean that no emphasis is given to other important aspects, such as etiology, which might serve as the basis for classification of subject-matter. In our procedure the emphasis is placed wherever it seems possible to bring out a principle. In some cases the stress may be put on lesion-type, in other instances on variety of symptoms exhibited, and in all cases the causal factor is examined as fully as possible. The primary division of the subject-matter, however, is the crop-basis.

An attempt to point out the advantages of such a plan raises the question of the objective. The aim should depend somewhat on what the student does after having had the course. The needs of the student rather than the whims of the teacher should be considered. The aim should also be determined, in part, by what best fits into the general curriculum of the college. An ideal, perhaps, is that the course shall attempt to reveal the relationship of plant pathology, a specialized branch of science, to knowledge as a whole. Finally, whatever may be the ultimate aims, the more immediate goal is to train the student, by means of plant pathology, in methods of scientific observation, thinking, and reasoning. In striving for this aim one is certain to teach something of plant pathology as a factor in agriculture and to enlist the sympathy and interest of students in our worthy field of study. The question then arises as to whether the method employed accomplishes these objectives.

In defense of the crop-basis method of organization, it may be said that a farmer is in the habit of grouping his problems by crops; crop production is his occupation. It is natural, then, that the agriculturist will think of his problems from the stand-point of the crop affected. He is interested

in the remedy. Remedial measures at times are directed against more than one pest affecting a crop. The important diseases of a crop can be arranged in seasonal sequence and a definite control-program can be offered.

Experience indicates that the student will return to the farm, either as a crop grower or as a rural leader. Therefore the beginning course in plant pathology should train him to recognize disease, to diagnose the more common diseases, and to help him understand the cause of disease. Such a training should lend support to his intelligent pursuit of any plant disease problem that may arise. If the problem is simple, he may solve it himself; if complicated, he will recognize that fact. In presenting the subject on a crop-basis plan, the plant disease problem is hitched to something already familiar to the student. On a basis of lesion-type or a causal agent, it is more difficult to make ready contact with the student's previous knowledge and experience. Suppose we divide plant diseases into necrotic, hyperplastic, etc., and approach the subject through these groups; or suppose we classify diseases on a basis of the causal factor, bacterial, fungous (and further, phycomycetous, ascomycetous, etc.) and then lead the student to plant pathology for his assimilation. In either case there is little in the student's previous experience that enables him to tie on to plant pathology, which for him is a new subject. No farmer ever started out to control bacterial diseases on the farm; but he does have interest in a program dealing with the diseases of a single crop. At the same time, however, there is no intention of denying the student a knowledge of the causal agent—a knowledge of which is essential to a thorough understanding of control, and is vital to an appreciation of the problem of disease in plants. In fact, the causal factor in each case is studied as fully as conditions will permit. Special attention is devoted to details of morphology and life-history of parasites. The final stress is then placed on those features of cycle and structure which bear on control. It is not that we minimize etiology; on the contrary we emphasize it. The general approach, however, is through crop groups. Of course, there are certain disease-types which group themselves somewhat naturally on an etiologic basis. For example, the mildews, smuts, and rusts constitute familiar groupings even in the lay mind, so that each of these types may well be treated together in an elementary course in plant pathology.

The course offered at Tennessee is general, covering the diseases of various crop-types. Chief emphasis is given to the more common diseases. It is felt that it is better to study thoroughly a few selected types than to attempt a review of a large number of known diseases of a given crop. With few exceptions, two laboratory periods are given to the study of a specific disease. Certain important diseases are necessarily omitted in such a scheme, but some of these are brought in for demonstration when the

control program for the disease of a given crop is under consideration.

The diseases which are selected for detailed study are those of chief importance in Tennessee. Some discussion is presented regarding certain diseases of national or even international importance in order to broaden the view of the student relative to the larger problems in plant pathology. I refer to such troubles as chestnut blight, citrus canker, white pine blister rust, potato wart and others.

The material actually employed in the laboratory exercises may be classified as fresh, dried, pickled; illustrations, cultures, and prepared slides. Dried and pickled materials are used in practically every study. In nearly every exercise, fresh materials are made available. Free use is made of illustrations; figures in books and bulletins, photographs—these are regarded as proper laboratory material. Sets of publications in particular, as far as available, are on file for the student's use as the need arises. Pure cultures of parasites are used wherever possible. The student is apparently more definitely impressed with the existence of an organism if it can be seen growing in mass separate from its host. Elementary spore-germination studies are made with the peach brown-rot fungus. This exercise is inserted in order that the student may better visualize incubation phenomena. Prepared slides are provided but to a limited extent and only after the student has knowledge of the source of the mount. These slides are used largely to facilitate study of details of fruiting body structure, and to aid in understanding pathological changes in diseased tissues (as in peach leaf curl). An abundance and variety of materials are aimed at, on the assumption that visual learning is best. Diagnosis, if it is to be learned at all in such a course, is learned from a large assortment of materials. Lantern slides are not available; when wisely used, they are doubtless of value.

Brief notice of the nature of laboratory procedure may be pertinent. After preliminary explanations are made, a difinite assignment of the exercise is announced. Time in the laboratory is largely spent by the student in a problem study of symptoms and in the more essential aspects of etiology. Drawings of chief features of the disease and parasite are made by the students. No time is given by the students to isolation of organisms nor to pure culture preparation; but enough practice in inoculation work is included to acquaint him with the general technique, the importance of, and the fact of, pathogenicity. Two field trips are on the program: one assumes the nature of a survey of the University farm, including cereals and fruit; the other of a vegetable-growing section nearby.

Written work is a valuable adjunct to the laboratory exercise. At the conclusion of each study, certain topics or questions are set forth. The student, with selected references to literature, writes on the topics as-

signed. The written work, together with the drawings made in the laboratory, constitutes the student's report on a unit of work.

One special report is required during the semester. This report is in the form of a longer paper, 1500 to 4000 words, which is compiled from the literature. The value in having the student go to the original sources is recognized. How literature originates, how to find it, its magnitude, its value, its use especially in so far as training is gained in sorting, writing, citing, editing, and the like—all these questions can be given attention in an exercise such as this. The student is asked to choose a subject, one pertaining to a plant disease which has not been included in the laboratory program; on this topic he is to write the paper. He is taught the nature and use of the Experiment Station Record as a source of references to literature. Later, other sources are introduced. When a reasonable number of references is accumulated, the student goes to the original publication, and the subject matter contained therein is abstracted. In making the abstract, the material is sorted, and the notes are made under headings definitely arranged in an outline. Abstracts are finally brought together and used in compiling the paper. The additional information on plant diseases and the peculiar training afforded by this type of exercise seem to justify the undertaking, alike in the eyes of instructor and students.

Thus the laboratory work is largely descriptive plant pathology. This is intended. The laboratory studies precede the lectures; this arrangement is also intentional. The lecture work deals to no little degree with analytical plant pathology: the "structure" of phenomena, the philosophy of the various aspects and recommendations, the inter-relationship of technical knowledge and practical application, the exact use and meaning of terms, and the interpretation of the more obvious truths. The lecture aims, then, at a ground-work which is necessary to an understanding of control-recommendations and practices. Usually most of the lecture hour is given over to a discussion of those features of a disease which are more definitely related to control; as a rule, however, the minimum time is actually devoted to details of the control measures themselves. An understanding of the principles on which control practices are based would seem more desirable than an extended discourse on methods of control. The methods, at least in detail, may change within a short time, whereas principles change more slowly. In each lecture, one point is stressed more than others; a theme properly selected and emphasized gives the student a frame-work to which subject-matter detail can be attached. In every instance, too, the important points regarding the various aspects of the disease are covered; these aspects are taken up in a definite order for each disease discussed and is practically uniform for all lectures.

No text-book is required. References to the best available literature are given out both at the lecture and in the laboratory. In some cases, where the

available literature is not suitable for student use, the lecture subject-matter is mimeographed and is distributed subsequent to the lecture. In such instances, as in many others, the students are tacitly advised against taking notes. This suggested procedure is based on the theory that taking notes results in a divided attention. I am inclined to the opinion that lectures and note-taking are over-done.

The so-called intelligence-test type of examination has been in use at Tennessee in the Department of Botany for a few years. The examination is divided into parts, or tests, as for example: true-false, selection, completion, matching, labelling drawings, and sometimes spelling. The advantages and disadvantages are probably known to everyone interested. While this kind of examination has proved useful and popular in our course, yet this type is not used to the exclusion of others. A few of the usual types of examinations, and frequent short oral or written quizzes are given. A final examination is given, but the emphasis is placed on daily work rather than on such a test.

In presenting any subject in a college curriculum, we should not lose sight of the student. The interest of the teacher should be in the student as well as in the subject. Sympathetic treatment of the student has a positive place in any course. It is advantageous to gain the student's point of view and criticisms; it has been said that the good teacher does not refuse to learn from his students. It is well to aim at the development of the maximum in the student's personality and character, but for the subject the aim should be to give the student only the optimum. The teacher's knowledge of his students should rate as high as his knowledge of the subject. Attempt should be made to give to the student "the spirit of learning, not learning itself"; and consideration should be given to the development of capacity, rather than to the stuffing of an already quite limited intellectual store-house.

Principles should be emphasized. Of course many facts are taught; it must be so! Facts must be stressed, but the final emphasis is put upon the principle which is synthesized out of the facts. Few rule-of-thumb formulae are taught; these can be had without taking the course. It is not enough to shower the student with an array of facts; principles must be developed. Nor is it sufficient merely to develop principles; these must be stated and restated.

The final objective is "not learning but rather discipline and enlightenment." We have no right merely to exploit the student to satisfy a hobby, but we should employ plant pathology to widen his vision; and, finally, we should use the course so that, along with other courses in the curriculum, it will function to develop the student—to increase capacity, discipline his mind, his habits, his point of view, reflections, discriminations, insight, perception.

UNIVERSITY OF TENNESSEE,
KNOXVILLE, TENN.

SOME POINTS OF VIEW ON THE PLANT VIRUS PROBLEM[1]

JAMES JOHNSON

There are ardent supporters of most, if not all, of the conceivable theories concerning the casual agency of virus diseases. This lack of agreement seems to be largely the result of the chaotic state of the knowledge of details involved in the separate phases of the problem. It is probably quite within the power of present research technique to determine these details definitely. While fundamentally important progress has been made since the recognition of the first virus disease, the data and conclusions have unfortunately been so conflicting as to point in no general direction. The difficulties have been, and apparently will be, increased in the future by the fact that nomenclature is based largely on symptomatology. While we have in general a fairly distinct set of symptoms for virus diseases, these are limited usually to chlorosis, mottling, malformation, necrosis and stunting, with intergradations which soon render impossible the selection of valuable descriptive names for the disease. It may soon be advisable, therefore, to abandon the attempt to describe a virus by the disease which it causes in favor of some other system more comparable to that used in the classification of bacteria and certain fungi.

SPECIFICITY OF VIRUSES

Transmission experiments and symptomatology have demonstrated that, in the potato virus group, a dozen or more apparently distinct viruses exist. The question of specificity of these viruses has been determined largely through symptomatology and through inter-varietal inoculations. While it has been reported by some workers that certain of these potato virus diseases are transmissible to other solanaceous plants, one is led to wonder if the viruses in question actually were transmitted, in view of recent results obtained in transmitting viruses with equal ease from apparently healthy and diseased potatoes to tobacco. In any case, the majority of the characteristic potato virus diseases do not appear to be readily, if at all, transmissible to certain other closely related plants. A similarly restricted host range probably exists in the case of certain other groups of viruses.

[1] Invitation paper read before the joint session of the American Phytopathological Society and the American Potato Association, Kansas City, Dec. 30, 1925. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, and the Chief of the Bureau of Plant Industry, United States Department of Agriculture.

On the other hand, Elmer's recent work would indicate that the virus employed was not specific as to species, genera, families or orders. Fifteen inter-family and eleven inter-order transmissions are reported, presumably on the assumption that one virus was used in all cases. A year ago only one virus disease of tobacco was recognized in our laboratory. At present we recognize nine or ten distinct viruses, if we include those secured from the potato. Symptoms, differential hosts, and other characteristics plainly point to the conclusion that these viruses are distinct, though admittedly not easily differentiated in some cases. The ordinary tobacco mosaic has probably not been transmitted outside of the solanaceous family. A specific virus may in some cases, however, pass directly from one family to another, as in the case of cucumber mosaic to tobacco.

Elmer probably was not working with a single virus but with several viruses, since he states that infected plants employed as sources of inoculum were obtained from widely separated localities, a fact which greatly reduced the chance that any specific cosmopolitan virus was used. His results are nevertheless very suggestive in the interesting field of virus transmission and host range. To be of most value, however, his experiments should be repeated with the question of virus specificity more clearly in mind; and future host range studies should take this consideration into account.

COMBINATIONS OF VIRUSES

It has been believed for some time that various distinct viruses may coexist in individual plants, especially in the potato. The potato viruses and their host range are probably not so well adapted to a study of combinations of viruses as are many of those which attack tobacco and other solanaceous plants. Certain combinations of viruses are apparently much more likely to produce a malignant disease than a single virus. With nine or ten viruses affecting as many or more host species, there is ample room for studying this interesting relationship. The separation of the viruses which are purposely combined, or the study of possible combinations found in nature, often is quite feasible by the use of differential hosts and other methods of separation such as aging *in vitro* and the selective killing action of heat and chemicals. Cucumber mosaic and tobacco mosaic in the same inoculum should be separable, for instance, by inoculating to pokeweed to get the cucumber mosaic free from tobacco mosaic. On the other hand, by permitting the inoculum to age for two or more days outside the living host, the tobacco virus should be secured free from the cucumber mosaic virus.

INSECT RELATIONS

The present status of our knowledge of the fundamental relation of insects to certain virus diseases is confusing and is likely to remain as a

barrier to clear thinking on the virus problem until clarified. The limited evidence which exists indicating that certain insects play a necessary or fundamental part in the life cycle of the virus concerned should be extended and corroborated if possible. Experiments indicating that an infectious disease can be transmitted only by insects or by grafting need to be performed with great care in respect to method of inoculation, the source of the inoculum, the condition of the host, the environment and other circumstances in order to be wholly convincing.

In the meantime, however, these insect relations should not be permitted to confuse us in regard to the majority of our more typical filterable viruses, for which we can be reasonably certain that insects are not essential, and that the part played by them is in all probability purely mechanical.

From an economic point of view it seems generally accepted that insects are largely, if not entirely, responsible for the occurrence of epiphytotics of most of the virus diseases. Following several years of observation and considerable experimental work, we have yet to be convinced that insects play a major role in the occurrence of epiphytotics of tobacco mosaic, although admittedly no good single substitute which is generally applicable as an explanation has been definitely discovered. Transmission through soil by the various means of contact and by storms may be much more common than generally supposed.

ENVIRONMENT RELATIONS

The relation of environment to virus diseases will require much more investigation than has been accorded to it up to this time to be adequately understood. Virus diseases are radically different from most parasitic diseases in that moisture does not play an important part in infection. This may be accounted for by the fact that infection occurs largely through wounds and that the virus almost immediately enters into close association with the surrounding protoplasm. The virus must either grow throughout the plant from the point of infection or be carried through it by normal physiological processes. Judging by its rapid systemic invasion, it is likely that its multiplication and diffusion occur simultaneously. Temperature is perhaps the only factor to which the virus diseases studied are particularly sensitive, but it is a disputed question whether this influence is exerted upon the virus directly or upon the host plant and hence indirectly on the virus. The expression of potato mosaic is most typical and marked at comparatively low temperatures (15° to 20° C.). Above 25° C. the symptoms are masked to a striking degree, not only on the newly formed leaves but also on older leaves previously showing distinct mottling. Furthermore it does not require a continued high temperature to bring about

this result, as shown in our laboratory by Tompkins; up to 80 per cent of the time may be spent at the low temperatures with a similar, though delayed, final result. The masking of symptoms already present seems most easily interpreted on the basis that the virus is reduced in activity in some manner by the high temperatures. While it is generally recognized by all workers with mosaic that the optimum temperature for the vigorous development of the host is the optimum for the shortest incubation period, this does not entirely explain the symptomatology in the potato. The potato plant may develop more rapidly at 25° C. than at 15° C.; yet the symptoms of mosaic as they develop from the tuber will be much more marked at 15° C. than at 25° C. On the other hand, if healthy young potato plants are inoculated with the mosaic virus, the plants placed at 25° C. for some days will show symptoms much earlier than plants kept constantly at 15° C. The virus apparently is not attenuated by the higher temperatures. In other words, a temperature favorable for masking is at the same time near the optimal temperature for infection as measured by the length of the incubation period. Again, if one is working with two or more different viruses on the same host in constant temperature chambers, a given temperature may be considerably more favorable for infection by one virus than by the other; or, on the other hand, a given temperature may mask one virus disease and not the other. Consequently the influence of temperature probably is more significant than a simple influence on host development.

SYMPTOMLESS CARRIERS

The possibility of plants carrying a virus without ever being capable of expressing any symptoms of the infection is an interesting and important problem. If this is actually true, it indicates a peculiar physiological relationship in regard to the nutrition and reproduction of the virus. In this connection, from a pathological standpoint a fundamental difference exists between masking and symptomless carriers. In the case of masking, it is necessary to assume only that the virus is in a dormant condition, or, as is more likely, the host is not in a physiological or morphological condition satisfactory for the expression of symptoms. In the case of symptomless carriers the virus must be assumed to be actively reproducing itself at the expense of the host without in any way appreciably affecting its appearance. However, recent results lead us seriously to question the existence of the reported cases of symptomless carriers. Certainly the original case of *Nicotiana glauca* cannot be left in this category, since this host may be markedly stunted by tobacco mosaic and strikingly malformed by cucumber mosaic. The cases in *Physalis*, as reported by Nishimura and by Elmer, are usually regarded as typical examples of symptomless carriers. Our experi-

ments with these species recently have shown that symptoms can be obtained under favorable environmental conditions. As I have pointed out in a recent article, however, the potato may eventually prove to be an excellent example of a symptomless carrier of viruses, since apparently healthy plants appear to be commonly infected. On the other hand, certain plants like *Nicotiana glutinosa* and the potato, which may be seriously affected locally by a virus, do not appear to carry the virus systemically.

PROPERTIES OF THE VIRUSES

This fertile field for investigation is too large to venture far for present purposes. It may be recalled, however, that tobacco mosaic is comparatively resistant to aging, putrefaction, desiccation, heat, and chemicals. Cucumber mosaic on tobacco produces symptoms so similar that the fact that these viruses are different has escaped detection for years. The main properties of the cucumber virus, however, apparently remain essentially the same in tobacco as in the cucumber. As a source of inoculum for mosaic Elmer recommends the use of vigorously growing parts of infected plants. Our experience with the tobacco mosaic virus in overwintering studies has shown that virus harbored on old boards or rags for a period of two years is practically as effective when extracted from these materials as inoculum extracted from green plants. Elmer, however, may have been using the cucumber mosaic virus or a similar virus, in which case his conclusion is probably correct, owing to the comparative sensitiveness of some viruses to adverse conditions. In this connection it may be mentioned that the host plant itself may have a marked influence upon the value of the virus for inoculum purposes. Pokeweed, for instance, is in our experience a poor source of cucumber mosaic virus for inoculation to certain unrelated plants as compared with tobacco. This may explain in part the apparent value of bridging hosts in some cases, rather than any actual change in the nature or properties of the viruses.

While tobacco mosaic and other viruses are surprisingly resistant to certain toxic chemicals, they may be often readily destroyed by such milder forms of chemical activity as represented by the action of other living organisms or by enzymes. For example, the virus is readily destroyed in moist soil as compared with dry or sterile soil. In slowly curing tobacco (presumably an enzymatic process) the virus may be fairly rapidly inactivated as compared with tobacco which is rapidly dried or kept as a liquid extract.

SIZE OF PARTICLES

There still seems to exist a remarkable difference in opinion as to the probable size of the particles concerned in filtrable viruses. While it is

generally recognized that fine grades of porcelain filters will prevent the passage of a mosaic virus and that coarser grades will permit the virus to pass, at the same time yielding a sterile filtrate, it is yet maintained by some that certain microscopic protozoa and bacteria are filter passers and that they are consequently not necessarily excluded from the category of probable causal agents. Others believe that ultramicroscopic fragments of these organisms, capable of acting as reproductive bodies, may pass extremely fine filters. Duggar and Karrer have already shown that the tobacco mosaic virus must exist in particles of extremely small size. Continuing this line of investigation, Mulvania, working in our laboratory, has repeatedly demonstrated that the tobacco mosaic virus will dialyze through certain graded collodion ultrafilters which, according to all probabilities, should not permit the passage of particles much larger than the ordinary protein molecule. One naturally wonders, therefore, if it is possible that the mosaic particles are living entities. There are those who would prefer to agree with Beijerinck that the virus is essentially a solution rather than a suspension, while still others prefer not to think of it as a virus at all in the sense that it reproduces itself, but suggest that the causal agency may be of the nature of an auto-catalytic agent. At any rate, the burden of proof as to the approximate size of the virus particles does not seem to lie with those who contend that the causal agency exists in extremely small particles. While certain protozoa and bacteria may pass through antibacterial filters bodily or fragmented, it is extremely unlikely that such bodies will dialyze through collodion membranes.

INTRA-CELLULAR BODIES

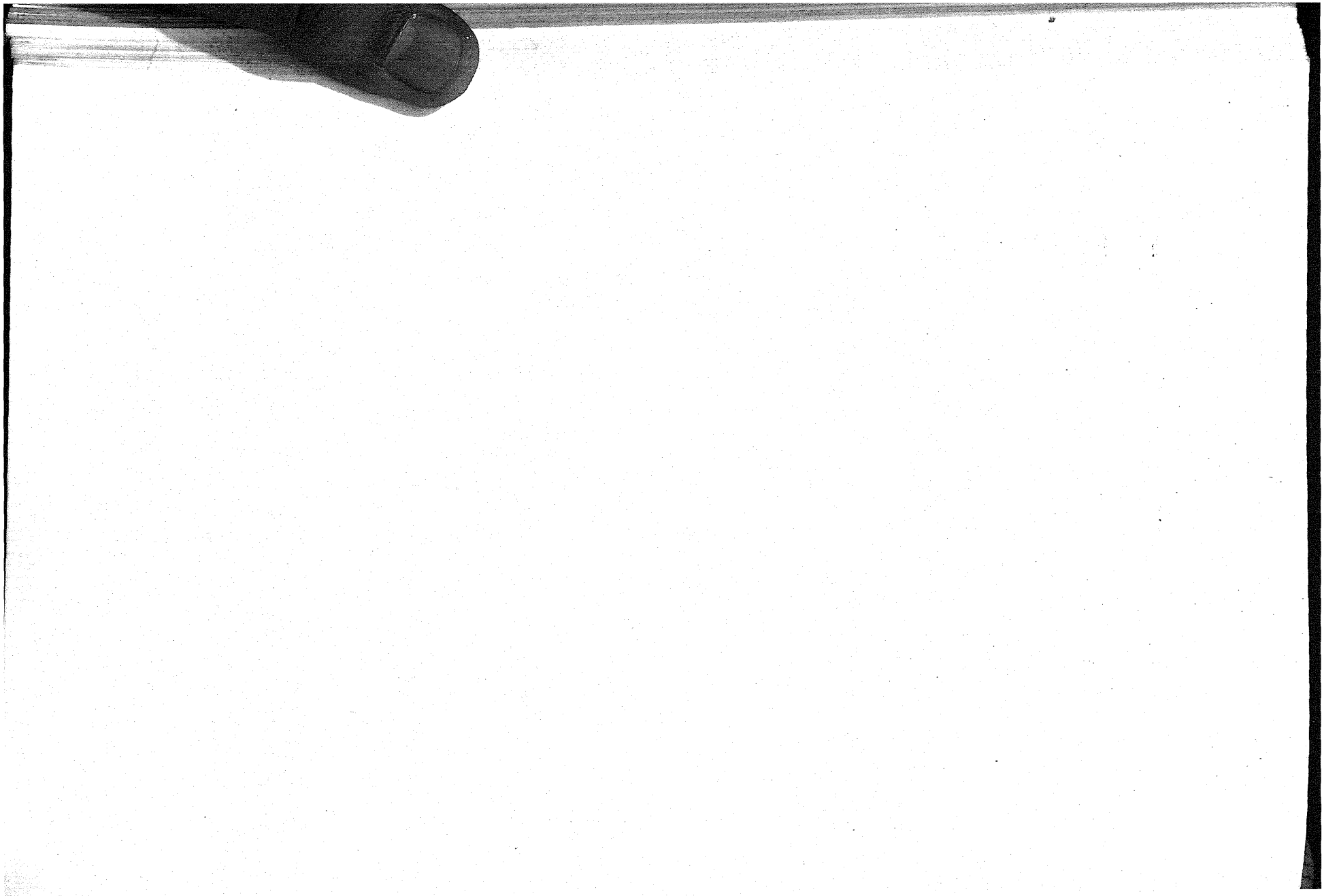
The plasmodium-like bodies, usually of surprisingly definite structure, and commonly associated with certain virus diseases in the host cells, remain puzzling. The proof of the causal relationship of these bodies to the disease has, however, always seemed insurmountable in view of the extremely small size of the infectious mosaic particles, unless a mode of reproduction heretofore unknown is assumed. To those who are still concerned with their possible causal relationship, it may be of interest to know that continued cytological observations by Miss Ismè Hoggan in our laboratory indicate that, while these bodies are readily found in a number of different hosts infected with the tobacco mosaic virus, they apparently are lacking in these same hosts infected with other virus diseases producing quite similar symptoms. These results suggest that no necessary relationship exists between the intra-cellular bodies and the occurrence of virus diseases. It is most probable that the intra-cellular bodies are a result rather than a cause of the disease. There seems to be some correlation, however, between the occurrence of these bodies and the ability of the virus to survive for long periods outside the living plant.

ARTIFICIAL CULTURES

Almost all investigators of virus diseases begin with an attempt at culturing the causal agency but are for the most part rapidly discouraged despite the number of partial successes reported. A year ago Olitsky reported on what appeared to be a good basis that the culturing of a mosaic virus had been accomplished. Mulvania tried to repeat this in our laboratories without success, and I understand others also have failed. For the present at least this phase of the problem also must remain unsettled, since negative evidence is rarely wholly convincing.

In conclusion, the results of the recent investigations on the viruses secured from apparently healthy potatoes may be mentioned briefly. The main results of this work have been published so recently that little important new data has been secured. It has been suggested by this work that either potatoes are exceptionally good examples of symptomless carriers or that normal or abnormal protoplasm of the potato itself is capable of attacking, as it were, the tobacco plant. I am indebted to Dr. H. H. Whetzel for the suggestion of the word "viroplasm" to cover the meaning of the latter working hypothesis. While this hypothesis may be weak from one point of view, it has the advantage of explaining practically all that is definitely known regarding the properties and behavior of plant viruses. However, one of these viruses of the type secured from some apparently healthy potatoes which we have referred to as "spot-necrosis" on tobacco is capable of causing a serious and even fatal disease when inoculated back to the variety of potatoes from which it came. The virus may remain dormant in the tubers for months, as in the case of other virus diseases of the potato. An explanation of this phenomenon on the basis of increase and decrease in virulence of the virus does not appear wholly to account for the facts. Something has occurred in this process of transference from one plant to another which suggests that we are working close to the boundary line of knowledge which so effectively screens from our view the actual nature of the causal agency of filterable virus diseases. If any significance at all can be attached to the viewpoints and experiments mentioned in this paper, they seem to suggest at least that the causal agency of virus diseases of plants is in some way closely related biologically to the living matter of green plants.

UNIVERSITY OF WISCONSIN AND
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE.



PHYTOPATHOLOGICAL NOTES

Factors affecting the properties of a virus.—Experiments carried out with tobacco mosaic show that virus of low concentration has a lower thermal destruction point than a concentrated virus. Diluted virus also loses its potency faster than concentrated virus on standing at room temperature.

When tobacco and cucumber juices were used as diluting media, the potency of the virus and its thermal destruction point was depressed more than when sterile distilled water was used for making dilutions. Cucumber juice had an especially marked depressing effect on the thermal inactivation point of the virus, and this juice also seemed more effective in reducing the potency of the virus on standing.

These studies suggest that the first reduction in the potency of diluted virus is not always permanent, as, in some cases, an increase in potency was found on the sixth and twelfth days after dilution. However, on standing 50 days, there seemed to be a decrease in potency below any previous low point. Additional study is necessary on this phase in order to distinguish positively between what may be experimental variation and reactivation of previously inactivated virus through colloidal and adsorption changes, or an actual increase in the number of virus particles not previously inactivated.—H. H. McKinney, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Wisconsin Agricultural Experiment Station, Cooperating.

Authority.—The following quotation furnishes the authority for, and the definition of, a very useful word in plant pathology—a word which either has not yet found its way into the dictionary or else is mentioned at the bottom of the page. The authority is the distinguished Belgian academician, Ch. Morren, who was perhaps the first to champion the mycetogenetic origin of potato blight. The complete citation is as follows: Morren, Ch. Des causes des disettes en céréales, depuis le commencement du XIX siècle. Bul. Acad. Roy. Sci. Bruxelles 20^3: 169–200. 1853. The article is reprinted in Morren's *Clusia,* Liége, 1852–1874, on the second pages (29) to (60). On page 174 [second page (34) of *Clusia*] one may find the following paragraph:

"On peut raisonnablement admettre qu'en prenant l'agriculture depuis le commencement du siècle, les sciences naturelles se soient assez perfectionées pour qu'elles aient pu faire une distinction entre les disettes produites par les phénomènes météorologiques et celles dont la cause dépend d'une véritable épidémie d'animaux ou de plantes nuisibles. Si l'on pouvait créer des mots à volonté pour rendre ses idées, le nom

d'*épizootie*, exprimant une maladie quelconque attaquant le bétail dans son ensemble, devrait trouver un mot correspondant à l'égard des plantes cultivées pour les récoltes. Le nom d'*epiphytotie* serait assez convenable pour exprimer ces maladies générales des plantes utiles. Ce serait alors à l'étude des causes de ces *épiphytoties* qu'il fraudrait se livrer.''

The statement is also of particular significance when it is recalled that, with the exception of Payen in France and Berkeley in England, the scientists of the time had been saying for the past nine years that *Botrytis* was the result of the potato disease, not the cause of it.—D. REDDICK.

ABSTRACTS OF PAPERS READ AT THE SEVENTH ANNUAL MEETING OF THE CANADIAN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, ST. CATHARINES, ONTARIO, DECEMBER 21 AND 22, 1925

Studies on the epidemiology of wheat stem rust in Manitoba, 1925. D. L. BAILEY and J. H. CRAIGIE.

Slides were exposed at various intervals from the first of July to the end of September by aeroplane at elevations of 3,000 to 5,000 feet on the Royal Canadian Air Force patrols at Victoria Beach, Norway House, and the Pas in Manitoba, and at High River in Alberta. When the results of these exposures were correlated with field observations, the indications were that rust spores had been carried by the wind for considerable distances from their place of origin.

Corrosive sublimate and time of treatment in relation to yield and control of Rhizoctonia. J. E. HOWITT and W. G. EVANS.

The results of six years' experiments in treating seed potatoes with different strengths of corrosive sublimate, and for varying periods of immersion, may be summarized as follows.

1. In Ontario, the strengths of corrosive sublimate and times of immersion usually recommended, namely corrosive sublimate 1 part by weight to 2,000 of water for 3 hours, corrosive sublimate 1 part by weight to 1,000 of water for 2 hours, and 1 part by weight to 1,200 of water for 1 hour, do not reduce the amount of *Rhizoctonia* sufficiently to insure satisfactory seed potatoes.

2. Much stronger solutions of corrosive sublimate than have been usually recommended can be used with safety if the disinfection is done very early in the spring when the potatoes are completely dormant; but, if potatoes are not completely dormant at time of disinfection, strong corrosive sublimate solutions cannot be used safely, as they retard the development of the sprouts and reduce the yield. In the experiments recorded, in a 5-year test, corrosive sublimate, of the strength of 1 part by weight to 500 of water for 2 hours, practically eliminated disfigured tubers, the average percentage for the period of 5 years being only 2.2 per cent. The average percentage in the check for a period of 6 years was 68.43 per cent.

3. Entirely satisfactory results cannot be obtained by disinfecting seed potatoes with any strength of corrosive sublimate after the eyes have started to grow. Strong solutions delay sprouting of the seed pieces and reduce the yield; and weak solutions, while they apparently increase the yield of salable tubers, do not sufficiently reduce the amount of disfigurement due to sclerotia to make the potatoes desirable for seed purposes.

Verticillium "wilt" of the raspberry. G. H. BERKELEY and A. B. JACKSON.

This disease was first reported by the authors in 1923 as "Bluestem." It is now considered advisable to discard this term for "wilt." The symptoms are a wilting and casting of the leaves, with an accompanying bluish discoloration of the cane. This disease is general throughout Ontario and has been found on Cuthbert, St. Regis, Marl-

boro, and Viking varieties. The disease is caused by a previously undescribed species of *Verticillium* which the authors have named **Verticillium ovatum** Berkeley and Jackson. A description is given.

The authors also point out that the blackening of cultures of *Verticillium* spp. is caused by sclerotia in some cases, whereas in others it is produced by resting mycelium. On this basis, the forms are divided into two groups: Group A, the resting mycelial type; and Group B, the sclerotial type.

Some observations on ascospore discharge and dispersal of conidia of Venturia inequalis (Cooke) Winter. J. E. HOWITT and W. G. EVANS.

Observations and experiments carried on for a period of six years indicate the following.

Apparently the average mean temperature of the months of January, February and March has more influence upon ascospore development and primary discharge of ascospores in the spring than has the amount of precipitation from April 1 to primary ascospore discharge.

The average mean temperature for January, February and March is more closely related to ascospore development and discharge than is the mean temperature of the days in April and May preceding primary ascospore discharge.

Free ascospore discharge takes place in many seasons at the time that the leaf buds are just bursting and the blossom clusters pushing up, emphasizing the importance of the delayed dormant and pre-pink sprays in some years.

Ascospores in Ontario may retain their vitality, at least until the eighth of August, and probably until a much later date.

The conidia may be scattered freely by wind from tree to tree in an orchard.

Some fungus foes of the flower garden. A locality list with notes. J. DEARNESS.

Aster: Rust—*Coleosporium solidaginis* (Schw.) Thum.; not severe.
Yellows—organism undetermined; destructive.
Wilt—*Fusarium conglutinans callistephi* Beach; destructive.
Carnation: Rust—*Uromyces caryophyllinus* (Schrank.) Wint.
Chrysanthemum: *Cylindrosporium chrysanthemi* Ell and Dearn.
Cosmos: Wilt—undescribed.
Foxglove: Leaf-spot—*Phyllosticta digitalidis* Bellynck; injurious.
Geranium: Wilt—*Bacterium pelargonii* Brown, or *B. erodii* Lewis; destructive.
Gladiolus: Dry Rot—undetermined.
Hollyhock: Leaf-spot—*Cercospora althaeina* Sacc.; injurious.
Rust—*Puccinia malvacearum* Bert.; disfiguring and injurious.
Honeysuckle: Powdery mildew—*Microsphaera alni lonicerae* (D. C.) Salm.
"Die-back"—not determined.
Iris: Root rot—probably *Bacillus carotovorus* Jones; destructive.
Leaf-spot—*Didymellina iridis* (Desm.) Tisdale; injurious.
Paeony: Blight—*Botrytis* probably both *cinerea* Pers. and *paeoniae* Oud.
Phlox: Powdery mildew—*Erysiphe cichoracearum* D. C.
Rose: Powdery mildew—*Sphaerotheca pannosa* (Wallr.) Lev.; mostly *Oidium*.
Black leaf-spot—*Diplocarpon rosae* Wolf; common and injurious.
Rusts—*Earlea speciosa* (Schw.) Arth; *Phragmidium disciflorum* (Tode) James; *P. americanum* Diet.
Cankers—*Diaporthe umbrina* and *Coniothyrium* are to be looked for.
Snapdragon: Rust—*Puccinia antirrhini* Diet. & Holw.; transplanted from greenhouse to gardens but not sure to survive winter.

Sunflower: Rust—*Puccinia helianthi* Schw.; disfiguring and injurious.
Sweet Pea: Root rots—*Thielavia basicola* (B. and B.) Zopf.
Tulip: Blight—*Botrytis tulipae* (Lib.) Hopkins.
Violet: Root rot—*Thielavia basicola* (Berk. and Br.) Zopf.
Leaf-spot—*Cercospora violae* Sacc. on the English violet.

The dry rot disease of gladioli. F. L. DRAYTON.

Repeated isolations made from diseased gladioli sent by growers, and intercepted at the ports of entry in shipments from Holland, yielded a fungus which proved pathogenic and was later found to be identical with cultures received from Dr. Massey, of Cornell University, as the fungus causing the dry rot disease described by Mr. Errett Wallace in an unpublished thesis in 1909.

The potential importance of this disease is shown by the results of field experiments in which an average of 80 per cent, and in some varieties 100 per cent, infection was obtained, with checks remaining healthy.

The premature death and decay of diseased plants in the field and the presence of lesions and other symptoms on the diseased corms are described.

The causal fungus is quite distinct from *Septoria gladioli* Pass., the cause of the hard rot disease. Numerous small sclerotia are produced in culture and on the base of the leaf sheaths in diseased plants, but no sports have as yet been found. The mycelium is intracellular in its development and is accompanied by a disappearance of the starch and the formation of a layer of cork cambium which isolates the lesions. The vascular bundles are sometimes invaded also and become black.

For the control of this disease formalin, mercuric chloride, Uspulun, and Bayer Compound were used as corm treatments and soil drenches without success. The following recommendations are made however:

1. The strict avoidance of land which has borne a diseased crop for further gladiolus culture; also care to avoid the transference of soil from diseased to clean land.
2. Corms having lesions should not be planted. To be sure of the absence of lesions, the corm scales should be removed, if possible, just before planting.
3. Plants showing signs of disease during growth or at digging time should be discarded, for, if the cormels from diseased plants get mixed with those from healthy ones, it is impossible to separate them, and they become a source of infection when planted.
4. When cleaning the corms, it is possible to detect diseased corms and eliminate them.
5. Healthy corms selected from a mixture of diseased and healthy ones should be soaked in 5 per cent formalin for 10 minutes before they are planted.
6. Rigid inspection of corms should be made at the ports of entry.
7. Inspection and certification of corms, when the development of industry demands it, would aid materially in the control of gladiolus diseases. No decided resistance was observed, but the Primulinus hybrids used in these experiments were more susceptible than the Glandavensis types.

The work is not complete, but is reported largely to direct the attention of plant pathologists and growers to the disease and the recommendations for control based on field and laboratory studies.

A preliminary note on Fomes pinicola Fr. and Pholiota adiposa Fr.—two heterothallic species of wood-destroying fungi. IRENE MOUNCE.

No cytological investigation has hitherto been made of the mycelium of *Fomes pinicola* or *Pholiota adiposa*. The absence of clamp-connections from monosporous

mycelia and their presence in compound mycelia resulting from the pairing of monosporous mycelia are the only criteria which have been employed in determining that these two species of wood-destroying fungi are heterothallic.

It has also been found (a) that *Fomes pinicola* and *Pholiota adiposa* are bisexual; (b) that in both species (1) monosporous mycelia either remain wholly sterile or produce imperfect fruit-bodies which have never shed spores; on the other hand those compound mycelia formed by the union of two monosporous mycelia produce normal sporophores. (2) Monosporous mycelia isolated from spores of fruit-bodies from different localities are mutually fertile.

New and unusual diseases and injuries of tobacco. C. M. SLAGG.

The Fusarium leaf- and stem-disease of tobacco has been noted in the seedbeds and fields in Kentucky, Wisconsin, Connecticut, and Massachusetts since 1918, but the damage caused is slight. The curly dwarf disease, characterized by the stunting, rosetting, and leaf spotting of affected plants, has been observed in Connecticut, Ontario, and Wisconsin since 1922. In extreme cases, as high as 20 per cent of plants have been found affected. The cause is unknown. Injury from the residual effects of a blasting material known as "borrowite" occurred on tobacco plants at Harrow, Ontario, in 1925. A type of stunting and malformation of large field plants in Ontario and Quebec in 1924 was found to be caused by attack by the common burdock borer *Papaipema nitella* Gn. During the growing season of 1925 in the Okanagan Valley, British Columbia, a type of irrigation injury was observed showing sunken, longitudinal stalk cankers, abnormal defoliation, and, in some cases, frenching symptoms.

Experiments for the control of onion smut. D. R. SANDS.

In some parts of the onion growing marshes in southwestern Ontario, smut is causing considerable loss in the crop. Formalin and Kalamit were tried at various strengths, the object being to get a solution that would give the best results with the minimum quantity of water. A determination of the moisure content of the soil (black muck) was not made, but at the time of seeding and for a long period afterwards, the soil was much drier than usual. Results are given in the following table.

TABLE 1.—*The effect of solutions of formaldehyde and Kalamit on yield of onions in Ontario*

Strength of solution		Gallons per acre	Yield per acre in bu.
Solution used	Water in gallons		
1 pt. formalin	16	130	568
do	12	100	540
do	6	50	450
1 pt. Kalamit	12	100	522
do	6	50	440
Check rows—no treatment			198

In all of the above solutions, about the same quantity of formalin and of kalamit was used per acre; hence it will be seen that the best results were obtained where the greatest quantity of water was used. Rows were 16 inches apart. The measure was the imperial gallon (10 lbs. water) and imperial pint (20 oz.).

TITLES AND ABSTRACTS OF PAPERS READ AT THE TENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, MILLS COLLEGE, OAKLAND, CALIFORNIA, JUNE 16–19, 1926

Transpiration as a pathological factor.—R. E. SMITH.

Observations on citrus diseases in South Africa.—H. J. WEBER.

Plant diseases in 1925.—R. J. HASKELL.

Cytology of root tips from sugar beets having the curly-top disease.—T. E. RAWLINS.

A degeneration of the pericycle cells and several rows of adjacent cells was regularly observed in root tips from sugar beets having the curly-top disease. The first evidence of degeneration was seen in the abnormal staining reactions of nuclei in the pericycle cells. This abnormality was apparently followed by the development of a coarse reticulum in the nucleus, the disappearance of a distinct nucleolar vacuole, the occasional division of the nucleole into several parts, and the appearance of a dense reticulate cytoplasm in the degenerating cells.

Several types of heavily staining cell inclusions were quite regularly found in the cytoplasm of these degenerating cells. Some of the inclusions were irregular in form, had a dense homogeneous internal structure, and assumed no regular position in the cytoplasm. Others were more or less spherical in form, had a heterogeneous internal structure, and were usually found at each end of the cell.

The amorphous nature of all of the observed inclusions indicates that they are products of the disease rather than causal organisms.

What is "sore-shin"?—MICHAEL SHAPOVALOV.

The beginning of the cotton growing seasons of 1925 and 1926 in the Southwest was marked by a severe outbreak of brown stem lesions on the seedlings. The injury had an appearance of typical "sore-shin," but isolations from the lesions showed that a great proportion of them was not due to *Rhizoctonia*. Specimens from a southern part of the San Joaquin Valley yielded cultures of *Rhizoctonia* as well as *Fusarium*. Farther north mostly *Fusarium* spp. were obtained. Material from the Palo Verde Valley and the Yuma section gave in part *Rhizopus* sp. and in part *Fusarium* sp. At Riverside most of the lesions were due to *Fusarium*. Other miscellaneous fungi have been obtained, but in less significant numbers.

This field survey and subsequent greenhouse inoculations indicate that the cause of cottom stem lesions may vary in accordance with the soil fungous flora in different sections and with climatic changes in the same section. The common name "sore-shin" may be convenient in designating a general group of cotton stem lesions similar in external characteristics, but it may readily lead to confusion if used to signify only the injuries caused by *Rhizoctonia solani*.

Further studies on avocado fruit decays.—WM. T. HORNE.

Studies of avocado fruit rots have been continued. The outstanding importance of *Rhizopus* spp. continues to be evident. A feature of the rot not previously emphasized is that the limits of decay are not well marked, so that affected fruit can not readily be trimmed and the sound part used. Rhizopus rotted fruit has been fed to guinea pigs at the rate of five grams per animal, and ten grams have been fed to a rabbit, without injury. A rather tough elastic rot has been produced by inoculation with *Pythiacystis citrophthora* S. and S. The rot is firmer, finer textured, less moist and slower acting than Rhizopus rot. The blue and green molds of citrus fruits [*Penicillium italicum* Wehm. and *P. digitatum* (Fr.) Sacc.] have not been destructive on avocado in our inoculations, but the blue mold of apples, *P. expansum* Lk., has caused a rot which advances less rapidly and is dryer in texture than on apples. *Sclerotinia libertiana* Fcl. and *Botrytis vulgaris* Fr. produce extensive wine colored rots on dark fruit in artificial infection, but *Scl. cinerea* (Bon.) Schrot. from apricot has not grown well on avocado fruit.

Notes on some tropical anthracnoses.—WM. T. HORNE.

Studies have been carried on at Berkeley and field observations have been made in Florida and Cuba on anthracnose of lime (*Citrus aurantifolia* Sw.), mango (*Mangifera indica* L.), and anonaceous fruits, including the anon of sugar apple (*Anona squamosa* L.), and the soursop (*Anona muricata* L.). The work of Clausen[1] on *Gloeosporium limetticolum* has been confirmed in that this fungus causes anthracnose on young tissues of lime but not on other ordinary citrus species. Limes were also inoculated with the other fungi mentioned in this abstract, but anthracnose was not produced by them. A *Colletotrichum* was isolated from young mango fruits from Florida and Cuba showing typical anthracnose. This fungus produced typical anthracnose on mango foliage in the greenhouse. Other *Colletotrichums* and *Gloeosporium limetticolum* failed to give typical mango anthracnose, though flecking and staining of wound edges were produced.

A *Colletotrichum*-like fungus was isolated from mature fruits of soursop. It is distinct in culture from the fungi mentioned above. We did not succeed in producing typical anthracnose on young foliage of cherimoya (*Anona cherimolia* Mill.), nor on soursop, though some injury was caused in young soursop plants under extremely moist conditions in several of the inoculations.

Mutation in fungi.—O. A. PLUNKETT.

A study of 39 species of fungi belonging to 18 genera has shown mutation to occur in four genera and seven species, giving rise to 17 mutants. The fungi were all grown from single spores and the purity of all cultures thus guaranteed. Mutations appear as sectors in a normal colony. Mutation is evidenced in Alternaria species in general colony characters; rate of growth; conidial production; conidial length, breadth, and septation; mycelial characters, color, zonation, and presence or absence of aerial mycelium. Two mutants derived from a single-spore culture of *Sterigmatocystis niger* differ from the original essentially in the size of the spore heads and the length of the conidiophores. Five distinct mutants were derived from a single-conidial culture of *Colletotrichum lagenarium*. These mutants differ in general colony characters; production and position of acervuli; mycelial characters, color, zonation, density of growth, sclerotia, and presence or absence of aerial mycelium. Five isolations of *Glomerella cingulata* from various sources gave three cultures which mutated and two which did not.

[1] Clausen, R. E. A new fungus concerned in wither tip of varieties of *Citrus medica*. Phytopath. 2: 217–234. 1912.

From these three strains six mutants were obtained, only two of which appear identical in culture. Mutation is evidenced in these races of *Glomerella cingulata* on agar in general colony characters; production of acervuli; production and kinds of perithecia; mycelial characters, color, zonation, and presence or absence of aerial mycelium. On apples the mutants differ in color of spot produced; general surface appearance of spot; nature of rot; rate of rotting; abundance and position of acervuli; abundance and kind of perithecia; abundance of aerial or surface mycelium.

All of the mutants are permanent in character and do not revert to the original form even when grown for a long time on their normal host. Certain apparent reversions occur in sectors of mutant colonies, but the whole colony never changes. Mutants may give rise to further mutations. Experimental attempts to produce mutation artificially and to secure new races of fungi by selection have failed. Modifications produced by environmental changes, poisons, modified media, etc., revert to the original form when grown again under normal conditions. Mutation is common in certain fungi in culture and possibly is responsible for new races and strains in nature.

Single-spore isolation simplified.—H. N. HANSEN.

Glass capillaries slightly greater in diameter than the spores to be isolated are filled by capillarity from a spore suspension made in warm nutrient agar. After a few trials a spore dilution giving from 1 to 4 spores to a tube 3 cm. long is easily made. The filled capillaries are then examined under the microscope and broken up according to the number and position of the spores observed. The resultant pieces are again examined to make sure that each contains one spore only, then picked up with forceps, immersed in alcohol to sterilize the outside and placed in the desired medium. By using solid rather than liquid medium the capillaries may be broken without disturbing the contents and sterilized without injury to the spores. If a culture from a single hyphal tip is desired, the piece of glass tube with the spore is pressed to the bottom of the medium in a petri dish, where germination may be followed under the microscope. When the germinating hypha has grown beyond the end of the tube, it is cut off and the glass removed. A single hyphal tip is easily obtained in this manner because of the microscopic size of the tube.

Transpiration of healthy and blighted tomato plants (Western yellow tomato blight).—MICHAEL SHAPOVALOV.

It has been shown previously by the writer that a close and direct correlation exists between the rate of evaporation and the progress of Western tomato blight. The environment, whether natural or artificial, which tends to reduce the rate of evaporation from atmometers and the rate of transpiration from the plants is likely to reduce also the percentage of blight. It may have been expected that varieties possessing a greater transpiratory power would be more susceptible to blight than those which transpire less, but this does not necessarily appear to be the case. Dwarf Champion, which is generally recognized as more resistant to Western blight than Stone, shows a higher rate of transpiration than does the latter. A few other varieties behave similarly.

The rate of transpiration of blighted plants progressively decreases as the disease advances toward its last stage, culminating in a pronounced yellowing and death of the plant.

The transpiration data have been obtained with growing plants by means of the cobalt-chloride paper method as well as with cut shoots in potometers.

Breeding lettuce for tip-burn resistance.—J. B. SCHERRER and T. E. RAWLINS.

Breeding and selection work was begun in an endeavor to develop a tip-burn resistant head lettuce.

Iceberg variety, while not immune, shows considerably more resistance to tip-burn than the New York variety. Iceberg and New York were crossed in an attempt to develop a variety having the desirable characteristics of New York and the tip-burn resistance of Iceberg. The red color of Iceberg was found to be dominant, all of the F_1 plants and approximately three fourths of the F_2 plants showing some red color. The F_2 plantings contained about one-half as many tip-burned plants as the New York planting, and most of the tip-burned F_2 plants were less severely injured than the diseased New York plants. Approximately 15 per cent of the F_2 plants were very similar to the New York variety in that they had firm heads, no red color, and the New York type of leaf texture.

Inheritance of resistance to Helminthosporium californicum in a cross between Chevalier barley, a resistant variety, and Abyssinian, a susceptible variety.—W. W. MACKIE.

Within the past few years a serious disease of barley, caused by a new fungus identified as *Helminthosporium californicum*, has appeared in California. The name rusty blotch has been given to it because of the large, rusty colored lesions appearing on the leaf blades. Among the hundreds of barley varieties examined, one, the Chevalier, a two-rowed variety, appeared immune. Crosses and reciprocal crosses between Abyssinian, a higher yielding, lodging resistant, but rusty blotch susceptible variety, and Chevalier gave plants free from rusty blotch in the F_1 generation, indicating the dominance of resistance. In the F_2 generation approximately one-fourth of the plants were attacked, with the remainder free from rusty blotch, indicating an inheritance of resistance to rusty blotch in a simple 3:1 ratio due to a single factor for resistance.

This species cross, Chevalier (*Hordeum distichum*) × Abyssinian (*Hordeum deficiens*), gave plants which segregated in a 1:2:1 ratio or one-fourth homozygous *H. distichum* plants, one-half intermediate or heterozygous plants, and one-fourth homozygous *H. deficiens* plants.

All three of these groups of segregates were found to include rusty blotch infected plants in approximately a 3:1 ratio, as was found for the F_2 group as a whole.

These ratios will be checked by plant-to-row tests in the F_3 generation.

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STUDIES OF CROWNGALL AND WOUND OVERGROWTH ON APPLE NURSERY STOCK[1]

A. J. Riker and G. W. Keitt

INTRODUCTION

As far back as records are available to the writers, it appears that a large percentage of apple nursery trees propagated by the root-grafting method have been affected by enlargements, or overgrowths, most of which ordinarily develop about the union of stock and cion. Much difference of opinion has existed both as to the cause of these enlargements and their effects upon the plants. Following the discovery by Smith and his coworkers (24, 25) of the causal relation of *Bacterium tumefaciens* Smith and Town. to galls on various plants, including the apple, the malformations so commonly found about the unions of root-grafted apple nursery trees have been rather generally attributed to the action of this organism, and the sale of crowngalled apple trees has been prohibited by law in many states. In their comprehensive accounts of crowngall, Smith *et al* (25, 26) report that *Bact. tumefaciens* occurs inside the cells of plants which they studied, stimulating abnormal activities which lead to the development of "primary tumors," "tumor strands," and "secondary tumors." While these papers contain no demonstration that "tumor strands" and "secondary tumors" occur in young apple trees, it has been widely assumed that such is the case, and that a tree once affected by crowngall is subject to the danger of recurring development of "secondary tumors." Recently, however, Riker (15, 16) in the United States and Robinson and Walkden (22) in England, working independently, have reported that, in the host plants which they studied, the crowngall organism was intercellular and that "tumor strands" appeared in their experiments only following infection in the region of a condensed bud where there was subsequent elongation of the internodes. Levine (7) has corroborated this work on "tumor strands." These contributions appear to show that crowngall infections are much more localized

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

in their development than was previously supposed, and that there is no convincing evidence to demonstrate that "tumor strands" or "secondary tumors" play any significant part in crowngall of apply nursery stock. In consequence of these and other developments, a reconsideration of many aspects of the crowngall question seemed desirable. A committee representing the American Phytopathological Society, the American Society of Horticultural Science, the American Association of Economic Entomologists, and the American Association of Nurserymen was appointed to consider the question of official nursery inspection for crowngall. This committee held a crowngall symposium in connection with the annual meeting of the American Association for the Advancement of Science at Cincinnati in 1923 [see Stewart, (29)]. Among the points emphasized at this symposium were the difficulty of diagnosis of malformations on nursery apple trees and the inadequacy of available information concerning many important aspects of the general crowngall problem.

Following the Cincinnati meetings a research project was organized[2] whereby coordinated programs were initiated at the Iowa State College of Agriculture and Mechanic Arts and the University of Wisconsin. This paper contains an account of the work in progress at Wisconsin, preliminary reports of which have appeared (17, 18).

STUDIES OF CROWNGALL

A better understanding of the details of infection of grafted apple nursery stock by *Bact. tumefaciens* and of the characters of the resulting galls appeared to be of first importance. Accordingly, a study was made of the mode of infection by this organism and the development of the galls in various stages until their decomposition. For experimental plants the Wealthy variety of apple was chosen, because it is reported by many nurserymen to show commonly a very high percentage of root knot. Unless otherwise noted all the experimental apple trees and apple grafts mentioned in this paper are of the Wealthy variety. It was hoped that by adhering rather closely to one variety the results would be more nearly comparable. The plantings were all made, unless otherwise noted, in the greenhouses and gardens of the Department of Plant Pathology of the University of Wisconsin. Material both under glass and out of doors was given daily attention to provide water, drainage, sprays, and whatever was needed for favorable growing conditions.

[2] This work, which is being administered by the Crop Protection Institute through a committee consisting of I. E. Melhus (Chairman), G. W. Keitt, and M. F. Barrus, is supported cooperatively by the American Association of Nurserymen and individual nurserymen, the Iowa State College of Agriculture and Mechanic Arts, and the University of Wisconsin, in collaboration with the United States Department of Agriculture.

The crowngall organism appears to be capable of utilizing several different types of injuries to the external parts of the apple plant as infection courts. This was determined in an experiment in which injuries were made on the cion below the ground line on 20 one-year-old trees by a series of needle punctures, on 20 more by knife cuts, and on 20 others by bruises produced by blows from a hammer. In each case the crowngall organism used in previous work by Riker (15) was promptly applied in suspension in sterile distilled water to the surface of the wound. The technique was such, therefore, that the bacteria were not carried into the tissue in making the wound. The trees, which were dormant and rather dry at the time of injury, were planted May 1, 1925, and harvested the following fall. When the trees were dug those wounded by needle punctures showed eleven trees crowngalled, five smooth, and four dead; those wounded by knife cuts showed nine trees crowngalled, three smooth, and eight dead; while those wounded by hammer blows showed eleven trees crowngalled, four smooth, and five dead. A higher percentage of infection would probably have occurred if the organism had been carried into the tissue by the injury and if the trees had been actively growing instead of dormant at the time they were inoculated. Several of the dead trees bore the remains of decayed galls on the underground parts. From these experiments it is evident that crowngall infection may take place through any of the types of wound provided.

Infection of apple by *Bact. tumefaciens,* in conformity with reports by Smith *et al* (25) and Riker (15) for other plants, has been observed to occur only through wounds. The fresh unwounded callus on apple grafts appears not to be a common avenue of entry for this organism. Experiments on this subject were performed in the spring of 1924, when 100 grafts were prepared under conditions designed to exclude *Bact. tumefaciens.* The cions and stocks were dipped for one minute in an aqueous solution of mercuric cyanide, 1–1,000, and the knife was dipped before each cut in an aqueous solution of mercuric chloride, 1–1,000. After being callused in clean sand, the grafts were planted in soil on which no crop susceptible to crowngall had been grown for a number of years and which had just been steamed by the inverted pan method described by Johnson (4). As soon as the steaming was completed the treated soil was enclosed by boards to prevent washing by rain water. Successive lots of 10 of these grafts were inoculated at 10-day intervals, beginning with the day of planting. The earth was removed so as to expose the callus at the union, care being taken to avoid injury. The fresh callus and the earth immediately surrounding it were irrigated with one-half cc. of a concentrated water suspension of the crowngall organism which was used by Riker (15) in earlier studies. The soil was then replaced. In this experiment as in others the

fresh callus was found to be somewhat resistant to wetting. In cases in which the inoculum would not adhere to the surface it was placed on soil which was then brought in contact with the callus. Control inoculations by needle punctures on tomato and apple showed that the culture employed was pathogenic to both and that the environmental conditions of the experiment were favorable for the development of crowngall. When the trees were dug in the fall 85 were living but no crowngall was found. At the same time the above planting was made 60 commercial grafts which had been wrapped with unwaxed thread were dipped in a water suspension of the crowngall organism and planted in soil which had been liberally sprinkled five days previously with a similar suspension. Only one crowngall was found in this lot of trees when they were harvested at the end of the first growing season. Control inoculations by needle punctures on both apple and tomato produced well developed crowngalls. These experiments suggest that the callus at the union of apple grafts is not commonly an open infection court for the crowngall organism.

The above experiments were repeated in the growing season of 1925, when, however, only commercial grafts were planted. They were placed in soil which had not been steamed and which was not inoculated. As before 60 grafts were dipped in a water suspension of the crowngall organism at the time of planting. When they were dug in the following fall, none of them showed crowngall. As in the previous year 100 grafts were employed in an experiment in which the soil was removed from the unions of successive lots of 10 plants, at 10-day intervals, and one-half cc. of a water suspension of the crowngall organism was applied to the callus tissue and the soil at each union. When these plants were dug in the following fall only two of them had produced crowngalls. An additional series of 50 grafts was planted. On the day of planting three grafts received one-half cc. of the type of bacterial suspension just described. At three-day intervals, additional successive lots of three received similar treatments. When the grafts were dug in the fall none of them bore crowngalls. With each series of these experiments check inoculations on tomato and apple showed the cultures employed to be pathogenic to both and the environmental conditions favorable for the development of crowngall (Pl. XXXVIII, B). These results corroborate those of 1924 and indicate that, under the conditions of this experimentation, no part of the uninjured tissue of young apple trees, including the callus at the union of grafts, was commonly an open infection court for *Bact. tumefaciens*.

Though many writers on crowngall appear to have assumed that the callus tissue on apple grafts commonly serves as an open infection court for *Bact. tumefaciens*, the present writers have been able to find no record of convincing experimental evidence of such relationship. Until such evi-

dence is adduced, therefore, this assumption appears to be unwarranted.

Attention was next given to the characters of crowngall in various stages of its development on nursery apple trees. The crowngalls used for this work were induced by inoculations with the organism used in previous studies by Riker (15). The material studied comprised approximately 500 galls of different ages on grafts and young trees grown both in the greenhouse and in the field. The characters of these galls were indistinguishable from those of additional galls which occurred on apple trees from commercial nurseries and which yielded the crowngall organism in isolation studies. The term crowngall as employed in this paper refers to the true bacterial disease as it appeared in this material. It will be found that the description of crowngall which follows applies to only a small percentage of the knots found on commercial nursery apple trees. Other knots which have been confused with crowngall are described in a later section of this paper.

The microscopic appearance of crowngall on apple is quite similar to that described by Smith *et al* (26) for the Paris daisy and by Riker (15, 16) for the tomato. The interior of the gall proper appears to be composed for the most part of comparatively soft tissue interspersed with varying amounts of vascular elements. In some cases the gall, though comparatively soft in the early stages of its development, may have woody elements formed to such an extent that it becomes very hard (Pl. XXXIX, E, F, and Pl. XLII, A, B). The terms soft gall and hard gall may both be accurately applied to malformations induced on apple by the crowngall organism. However, the soft gall is the more common type. Regions of hyperplastic and hypertrophic tissue are readily found, but the more extreme types of the latter, as seen in the more succulent tissue of tomato and tobacco, did not appear in the apple material studied. In some places small centers of rapidly dividing cells may be seen which appear to be initiating new regions of hyperplasia. Cells with walls which are thickened in the manner of xylem tissue sometimes appear as isolated units and at other times they seem to approach the organization found in vascular bundles. In places, in connection with these woody elements, the arrangement of certain cells has suggested medullary rays, cambium, and cortical elements. In some of the galls structures were observed which gave the appearance, within a limited area, of several layers of cambium arranged at different distances from the surface (Pl. XLII, A, b, b′). The gall appears to enlarge by growth within the softer tissues beneath the surface, rather than by the callus type of growth (Pl. XLII, D, E), as described on a later page, or by the type of secondary thickening that follows cambial activity. The structure of the gall varies considerably at different stages of development and in different parts. It is common to observe tissue in the gall which is quite similar to callus and wound tissue. It has already

been suggested that a very close relationship exists between the pathological developments stimulated by the crowngall organism and those by various chemicals (27) and injuries (20). The surface of the gall appears not to consist of any specially differentiated tissue. It is quite common to find the outer cells dead (Pl. XLII, A, a).

The gross appearance of crowngalls (Pl. XXXVIII, A, B, C) is in close conformity with the characters observed under the microscope. The surface, which is comparatively smooth like that of crowngalls on peach and raspberry, is frequently covered by dead cells. A definitely organized cork layer has not been found on the gall proper but it may occur in connection with wound tissue wherever the crowngall is associated with wound overgrowth. However, the dead cells on the surface sometimes resemble cork. The granular, frosty appearance of the surface of fresh callus is not common and when it does occur it is usually limited to rather small areas of the surface of older galls. On the larger galls it is frequently found that certain portions have begun to decay. Small roots or root primordia seldom if ever originate from the crowngall proper. In cases in which roots have been observed in association with a crowngall they have been found to occur in connection with wound overgrowth that was adjacent to the gall. Wound tissue has been found in such position on a small percentage of the trees as if in response to an injury produced by the tumor. This is in conformity with a report by Muncie (11) that an infectious type of hairy root may arise from a gall. The gall tissue, as Smith *et al* (25) have reported, contains more oxidase than cortical tissue and turns brown more quickly upon exposure to the air. Crowngalls on the above ground portions of apple trees have been found to be smaller and harder than those below the soil line. This is probably due largely to the drying influence of the air as shown by Riker (19) on raspberry.

The characters of crowngall are sometimes obscure, especially on material collected in the nursery, because crowngall proper and excess callus or wound overgrowth, which are described later, may merge in a common enlargement. Crowngall may also occur from infection of callus or wound overgrowth. Since all of these overgrowths are initiated in wounds and are somewhat similar in gross characters, it is not surprising that they have been confused.

The identity of the crowngalls employed in these studies was checked by demonstrating through numerous isolation and reinoculation tests that they contained the crowngall organism. *Bact. tumefaciens* has been found to be continually present in undecayed portions of crowngalls at all stages of their development. Some of the galls studied were of considerable size and were doubtless several years old. The details of isolation technique and the number of enlargements examined are given on later pages in connection with investigations of wound overgrowths.

Difference of opinion exists with regard to the seat of the stimulus which incites the development of crowngall. The following facts suggest that the bacteria within the gall, rather than those on the surface as reported by Robinson and Walkden (22) for daisy, furnish the stimulus causing the development of crowngall on apple: (a) *Bact. tumefaciens* was isolated consistently and in abundance from the interior tissues of known crowngalls, (b) growth of crowngalls occurs primarily from the interior rather than the surface cells, and (c) the presence of the causal organism on the unbroken surface of host parts, even on fresh callus, did not induce gall development in the experiments reported above.

Galls induced by the crowngall organism appear under certain conditions to be subject to decay by secondary invaders. As reported above, a considerable number of the galls resulting from inoculations on grafts and one-year-old trees showed various stages of decay when they were dug in the fall. This fact is not surprising, since crowngall tissue is commonly soft and unprotected. It has been frequently observed in storage that, under conditions of comparatively high temperature and moisture in the spring, the rot organisms attacked the cortex of crowngalled trees in such manner as to girdle them. This type of injury has been observed to occur also on crowngalled trees in the field. Injury of this character was responsible for the loss of many of the inoculated grafts and trees in the greenhouse in two separate Wisconsin temperature tank (Jones, 5) series set up to determine the influence of soil temperature and soil moisture on the development of crowngall on apple. Practically all the apple trees at temperatures above 20° C. and at the same time in soil with a high moisture content were killed in this way. On the other hand, crowngalls have been kept very well in storage under conditions of low temperature and low moisture. Crowngalls from the previous year have been found in excellent condition on both apple and raspberry in the field in Kansas during the month of March and in Wisconsin during May. In some cases where the gall was badly decayed new gall tissue has been found to grow from the margin. Cultures of the crowngall organism have been secured without difficulty from these several types of specimens. So it is evident that, although crowngall on apple is quite subject to decay, it can persist from one growing season to the next on the host plant and thus supply a new potential source of inoculum after the winter.

Injury incident to crowngall infection of young apple trees has been observed to lead to unsatisfactory growth or death. The constriction-like effect described by Levine (6) and the reduction in water flow shown by Muncie (12) are probably factors in the inhibition of growth. However, the most serious source of injury probably results from the rot induced by secondary invaders. It appears that sooner or later bacterial crowngalls are

largely destroyed by such invaders, which are likely also to attack the cortex of young trees and induce girdling.

Various antiseptics were tried in the early phases of this work in an effort to control crowngall on apple. Among those employed were Bordeaux mixture, copper carbonate, lime-sulphur, sulphur, dry lime-sulphur, slaked lime, bleaching powder, lithium carbonate, sodium silicate, zinc chloride, ammonium hydroxide, potassium permanganate, ferric chloride, ferric sulphate, aluminum chloride, tannic acid, furfural, toluol, carbon bisulphide, laundry soap, and several organic mercurial compounds. Before they were tried on apple grafts in the field an idea of their toxicity to plants and their efficiency against crowngall infection was gained by tests on tomato plants in the greenhouse. These experiments indicated that with the materials used treatment previous to inoculation was of comparatively little value, probably in part because the wounds which were made to permit the entrance of the bacteria broke through the covering provided by the antiseptics. Treatment subsequent to inoculation was effective only when applied within a short time and then only where the antiseptic killed the cells that formed the infection court. About 11,000 grafts were treated in the field in lots varying from 60 to 200. In some cases the cions and roots were treated before the grafts were made. The antiseptics were applied only to grafts which were wrapped with waxed thread. The grafts were treated by dipping them into solutions, by dusting them, by planting them in sprayed and also in dusted trenches, and by puddling them in soil which had been treated with various antiseptics. These experiments were conducted for the most part at the North Star Nursery at Pardeeville, Wisconsin. The details of this long series of experiments are omitted because no satisfactory control of the malformations dealt with was secured.

A commercial preparation of hydroxymercurichlorophenol gave less promise of control of crowngall than some of the other compounds when tried against experimental inoculations. This was tested first on tomato plants. Twelve tomato stems were punctured five times each with a sterile needle. The first three plants were dipped in a solution of the commercial product, 1–400, and after an interval of a half-hour received a water suspension of the crowngall organism. In all the inoculations of this series the bacteria were applied with a camel's hair brush. The stems were still wet with the hydroxymercurichlorophenol solution at the time the bacteria were applied. The next three plants received the same treatment except that the bacteria were applied immediately after the plants were dipped in the solution and while the stems were well covered with it. The next three tomato plants received the application of the bacterial suspension promptly after wounding and were then dipped in the solution after an interval of less than one minute. The last three plants received the same treatment

except that a half-hour elapsed between the application of the bacteria and the treatment with the solution. The plants were all placed in the greenhouse under favorable conditions for the development of crowngall. The chemical produced no visibly injurious effect upon either the tomato stems or leaves. After three weeks the 12 tomato plants each showed five well developed crowngalls. This experiment casts doubt upon the value of hydroxymercurichlorophenol as a direct preventive of crowngall infection. A similar experiment was tried with another commercial preparation of hydroxymercurichlorophenol manufactured by a different company. Like results were obtained.

When apple grafts were dipped in a solution of hydroxymercurichlorophenol, 1–400, it was noticed that the fresh young callus became somewhat brown. This discoloration appeared to a less extent following dipping in water, and in a still smaller degree after exposure to the open air. The injury from several mercurial preparations was considerably more conspicuous when they were employed in the form of a dust. Many other chemicals used produced considerable injury to the fresh callus.

The possibility of controlling infection of grafted apple nursery stock by the crowngall organism with antiseptics appeared still less promising when it was learned that the fresh callus, the part which it was sought particularly to protect, probably is not ordinarily an open infection court for *Bact. tumefaciens.* Furthermore, as the experimental work progressed, it became evident that the great majority of the malformations encountered below the soil line on grafted apple trees in the nursery, aside from the well known enlargements caused by woolly aphis and nematodes, were not caused by *Bact. tumefaciens.* Consequently, it became necessary to shift the major attention from crowngall to these other malformations. Several recent reports of the control of "crowngall" by antiseptics are discussed on later pages.

STUDIES OF CALLUS AND WOUND OVERGROWTH

Since the malformations found on most of the root-grafted apple trees rejected as crowngalled at the nurseries had different characters from galls produced on young apple trees by inoculations with cultures of *Bact. tumefaciens,* an attempt was made to determine their nature, cause and control.

Superficially, these enlargements resembled the bacterial galls. However, closer examination in their various stages of development revealed distinct differences. The first stages observed were callus developments about wounds. In the early stages, growth of this callus occurred chiefly through the division and enlargement of the surface cells (Pl. XLII, E; XLIV, B; and Fig. 1). This tissue, which had a white, frosty appearance when fresh, was somewhat resistant to wetting but very susceptible to injury from

various chemicals, or even from exposure to the open air. On later pages these early stages of development are referred to as callus. While such callus tissue was occasionally found on parts of overgrowths which were as much as three years old, more commonly a phellogen was differentiated in the first or second year and a cork layer formed. From this time growth of the knots appeared to result chiefly from the activity of a cambium layer. Their interior was commonly, but not always, very hard, due to the presence of wood. Indeed, it was often more difficult to cut with a knife than the stem proper because of the irregularity in arrangement of the woody elements. This interior tissue appeared to contain much less oxidase than that of crowngall (Pl. XXXIX, E). In the succeeding pages, in view of evidence which will be presented, these malformations in this stage are called wound overgrowths. These enlargements were often very irregular in contour, due partly to the development of root primordia and roots. Zimmerman (34) has reported similar developments on hard-wood cuttings. Not infrequently a considerable number of roots grew out and produced the appearance of the common "woolly knot" (Pl. XXXIX, D). In some cases very

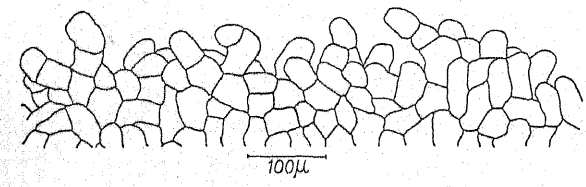

FIG. 1.—Section at the surface of fresh young callus on a Wealthy apple graft. The cells at the surface were alive and actively dividing. Drawing made with the aid of a camera lucida.

peculiar forms were produced by abortive and fasciated root development, which had some resemblance to balls of knotted string. Sometimes, as in Plate XXXIX, A, and XL, C, D, the diameter of the main axis below the enlargement was less than that above. As has been mentioned before, cases occasionally were found in which wound overgrowths occurred in combination with crowngall.

In view of the fact that the malformations just described have commonly been supposed to be crowngall, a bacteriological study of these enlargements was initiated. A standard technique was adopted with the aim of securing the maximal feasible accuracy and consistency from the method in the hands of several operators. The writers were very ably assisted in these isolation studies by Paul Miller, Ismé Hoggan, L. W. Boyle, and W. M. Banfield. Each of the individuals who performed this isolation work practiced on known crowngall material until he was able to isolate the causal organism consistently. Only after the technique was well mastered was he permitted to work on this project.

The procedure in the isolation work during the winter of 1924–1925 was as follows. The culture medium employed was composed of 17 gm. of agar, three gm. of beef extract, 10 gm. of peptone, 10 gm. of dextrose and 1,000 cc. of distilled water. The medium was prepared in the usual manner and adjusted to pH 7.0. The surface of the malformation to be examined was thoroughly cleansed with water. A portion of the interior was exposed with knives which were dipped in alcohol, flamed, and manipulated in accordance with the usual technique employed for securing interior tissues free from external contamination. By proceeding in this manner a portion of tissue was secured which was nearly or entirely free from surface contaminations. In the early phases of the work the surface of the gall was treated with mercuric chloride, 1–1,000, but this practice was soon discontinued because it appeared to reduce the chances of securing the crowngall organism. After a small bit of tissue, approximately one-sixteenth to one-eighth inch cube, was exposed under the conditions just described, it was removed to one cc. of sterile distilled water in a sterile petri dish where it was crushed or cut into very small portions. By this procedure fragments were removed from at least five different representatively distributed parts of the knot under examination including outer and inner portions, a separate petri dish being used for each portion of tissue cultured. After the crushed tissue had stood in the sterile distilled water approximately one-half hour to permit the diffusion of bacteria the plates were poured in the usual manner. At one time nutrient broth instead of distilled water was employed, but under the conditions of this work there appeared to be no advantage in using broth. The plates, which were incubated at approximately 22° C., were examined at frequent intervals and puncture inoculations into tomato stems were made from colonies which most resembled those of *Bact. tumefaciens*. Similar inoculations were also made from colonies of various types. For possible future study, transfers to agar slants were made from many of the colonies chosen for inoculation. These colonies were usually selected on the fourth or fifth day. However, no plates were discarded without being examined after they were at least 10 days old. Check inoculations with a known culture of the crowngall organism were made with each series. All the inoculated and control plants were kept, appropriately segregated, on a greenhouse bench where the temperature was approximately 22° C. and other conditions were favorable for the development of galls. Although the tomato plant was always employed, inoculations were also made from time to time on tobacco and geranium. With the various cultures used, these plants consistently yielded results which conformed with those on tomato. Ordinarily under the conditions of these studies the galls developed from inoculations with *Bact. tumefaciens* on tomato so as to be visible in seven days and conspicuous in ten days. However, the results

were never recorded as negative until an examination was made at least a month after the inoculation. The results on herbaceous plants were checked by inoculations on apple. Twenty-two inoculations on apple were incubated for two months, and 50 more on apple were incubated for an entire growing season. At the end of these periods all the trees were free from any enlargement, except those inoculated with the cultures which were positive on tomato. In every case the original knot cultured was kept and numbered in case further examination might be desired. Records which included entries under forty-nine different heads were kept for each knot examined. A sample of the record sheet is omitted because of its size. The writers understand that this technique is similar to that employed by Brown (1) in her work on the apple stem tumor. All cultural examinations referred to on later pages were made by this technique or the slightly modified method used in 1925–1926.

The efficiency of the isolation technique was tested at frequent intervals. For approximately every five trees cultured a check isolation was performed on crowngall of apple induced by inoculation or on crowngall of peach, cherry, or raspberry from natural infection. In 1924–1925 positive results were obtained from 44 of 46 such crowngalls. In this work, it was very common to secure the crowngall organism from three or four of the five pieces of gall selected for plating. In general practice it was found that on the average the crowngall bacteria were secured from approximately seven of every ten of individual pieces tried from known crowngall material. Consequently five separate attempts were made from each knot because it was desired to maintain a high degree of accuracy in the technique employed. It is considered very important that numerous checks should be made on the technique of isolations, because otherwise certain errors might creep in and reduce the accuracy of the studies.

Attention is invited to several other points in the procedure at which error may enter unless due precautions are taken. (a) It appears to be necessary for success in making isolations that the material should be fresh and the portion used should be free from decay. Two dozen attempts at isolation from decayed crowngall tissue have given uniformly negative results. A consideration of what becomes of the crowngall organism in decayed tissue is beyond the scope of this paper. (b) When inoculations are made on tomato plants to test the pathogenicity of the different cultures precautions should be taken to prevent the bacteria from being spread from one inoculation to another when the plants are watered. The possibility of this means of transfer was tested by placing a half dozen potted tomato plants inoculated in 52 places among a half dozen plants which were wounded by needle punctures in 230 places but uninoculated. All the plants, which were placed side by side on a greenhouse bench, were then watered with a

hose in the usual manner. After three weeks infection was found at 92 per cent of the punctures on the inoculated plants and at 21 per cent of the punctures on the wounded but uninoculated plants. (c) Another possible source of error is in the manner of making injuries for inoculations. This is especially important with woody plants, as apple, but is also important with some of the more herbaceous plants. The resemblance between crowngall and wound reaction on tomato has been discussed by Riker (20). In cases in which large wounds were made in woody plants, but no inoculum applied, small rather hard enlargements were sometimes produced after a considerable period. These resulted from the wounding, without the intervention of *Bact. tumefaciens*. Such overgrowths, however, might be confused with crowngall if not understood. These experiments are described on later pages. Meanwhile, it should be observed that infection with *Bact. tumefaciens* may be obtained consistently without making large wounds. (d) Certain other types of reaction which appeared to be associated with bacteria, but which, however, are not typical of crowngall, deserve more detailed consideration.

The interiors of the wound overgrowths on the apple material cultured were found sometimes to be sterile and sometimes to contain various types of bacteria. In agar plates some of these organisms produced colonies that resembled those of *Bact. tumefaciens*. The presence or absence of the different bacteria varied with the organization of the woody and softer elements. In places in which the usual type of cortex, as it occurs in apple stems or roots, covered a regular formation of xylem elements, bacteria very rarely were found. However, they often were isolated from places in which the wood and soft tissue elements were very considerably gnarled and twisted (Pl. XL, B, D and Pl. XLII, C). In such locations it was not uncommon to find small particles of soil which had probably been incorporated in the tissue as it enlarged in close contact with the soil. Different organisms from such regions showed a wide range of growth characters, fermentation reactions on sugar media, and dye absorption. The details of these experiments are omitted from this paper because the studies are incomplete. When the bacteria found in the wound overgrowths were inoculated into tomato the wounds in most cases corked off in the same manner as wounds made by puncture with a sterile needle. Such wound response in tomato has been observed to be comparatively small except at times in soft tissue near the top of the stem. In this position it has sometimes produced an enlargement which attained an elevation of one or two mm. in a month. A few of the bacteria isolated from wound overgrowths, however, appeared to induce certain reactions, three of which deserve mention. (A) In certain instances various degrees of rotting of the tomato stem tissue about the point of inoculation have resulted. The more extreme types suggested the

rot induced by *Bacillus carotovorus* Jones on carrot. Such a reaction has not been observed to be induced by *Bact. tumefaciens.* (B) In some cases the type of development just mentioned was followed several weeks after the inoculation by a slight enlargement. This was ordinarily quite small in comparison with crowngalls of the same age. (C) In some other cases rather similar swellings in the tomato stem tissue at the point of puncture were noticed where no rotting of the tissue occurred. These were very small in comparison with crowngalls of the same age which were induced by a known culture, being only slightly larger than the swelling which sometimes follows the puncture with a sterile needle. Similar swellings have been noted occasionally to develop about wounds in which either steamed or unsteamed soil had been placed. They suggest some of the swellings which were induced by Smith (27) with various chemicals. The identity of the organisms that appear to be associated with these enlargements has not yet been determined. In view of the facts that (a) they were secured from knots on apple which did not resemble those induced by known cultures of the crowngall organism, (b) in some cases their cultural characters were different from those of this parasite, and (c) the reaction which they induced on tomato stems was different from that induced by this organism, the question is raised as to whether or not they should be called *Bact. tumefaciens.* It appears that considerably more evidence of every kind will be necessary before these organisms can be identified. They may be either strains of the crowngall organism or distinct from it. They have been found in less than ten per cent of the knots examined. The organisms which were isolated from the interior of wound overgrowths, but which induced on the tomato stems no swelling larger than wound reactions to the inoculating needle, have not been identified. When placed in needle punctures they have failed to induce any suggestion of crowngall on tomato, geranium, tobacco, or apple. It is not surprising that the colonies of some of these bacteria on agar plates resembled those of the crowngall organism, since the distinguishing characters of bacterial colonies on agar plates are comparatively few and there are many kinds of bacteria in the soil. It seems probable that these bacteria were soil organisms which gained entrance to the tissues of the wound overgrowths and survived.

Smith *et al* (25) report differences in cultural characters of certain strains of *Bact. tumefaciens.* Limited studies of this subject by the present writers have yielded similar results.

For the isolation studies of 1924–1925 approximately 1,500 root-grafted apple trees which were representative of those rejected as crowngalled were secured from twelve of the leading nurseries in the states of Wisconsin, Iowa, Nebraska, Kansas, Missouri, and Tennessee. To these were added approximately 500 trees from plantings at Madison, Wisconsin. By means

of the technique described above, an examination was made of the overgrowths of 227 of these trees which were selected at random to include representative specimens from each lot. The trees were of various sizes and ages. Of the total number 103 were less than one year old, having been collected at intervals from the day of planting to the end of the growing season. Of the 227 malformations approximately two per cent had the characters of crowngall as described above. These two per cent readily yielded bacteria which, when inoculated into tomato, geranium, tobacco, or apple induced crowngalls indistinguishable from those caused by known cultures of the crowngall organism. Approximately eight per cent more of the malformations yielded organisms which, when inoculated into susceptible plants, induced slight swellings which were much smaller and slower to develop than those induced by *Bact. tumefaciens*. These eight per cent possessed either the characters of wound overgrowths or a mixture of those of wound overgrowths and crowngall. The relationship of these bacteria to the enlargements from which they came has not been determined. The other 90 per cent of the enlargements examined had the characters of wound overgrowths. The interiors of these enlargements were either sterile or yielded organisms that did not produce any evident reaction upon tomato, geranium, tobacco, or apple. The evidence from these studies strongly suggests that the crowngall organism is responsible for only a comparatively small portion of the malformations on the nursery apple trees studied.

In the season 1925–1926 the results obtained were similar to those of the previous year, except that a slightly higher percentage of crowngall was found. The material cultured was obtained from 15 nurseries in the states of Arkansas, Illinois, Iowa, Kansas, Michigan, Minnesota, Missouri, New York, Pennsylvania, and Wisconsin. Most of these nurseries were different from those which supplied the trees used in the season 1924–1925. The isolation technique was varied from that used the year before by employing a medium similar to that used by Wright (33). A 10 per cent stock solution of yeast water was prepared by placing 500 grams of starch-free yeast in 5,000 cubic centimeters of water, steaming for two hours, and autoclaving for one hour at 15 pounds pressure. After the insoluble material had settled for several days the clear supernatant liquid was used. The medium was made of the following components:

Stock solution yeast water	100.0 cc.
Distilled water	900.0 cc.
Di-basic potassium phosphate (K_2HPO_4)	4.0 gm.
Sodium chloride (NaCl)	.2 gm.
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	.2 gm.
Calcium carbonate ($CaCO_3$)	3.0 gm.
Dextrose ($C_6H_{12}O_6$)	10.0 gm.
Agar	20.0 gm.

Reaction adjusted to pH 6.8

Examination was made of malformations on 156 trees. These were taken at random except that no enlargements were included which were comparatively small in relation to the diameter of the tree. Eleven per cent were positive for crowngall, 8 per cent were doubtful as described earlier, and 81 per cent were negative. In addition to these specimens, 24 more were selected for culture because they showed characters of both wound overgrowth and crowngall. Of this number 13 per cent were positive for crowngall, 25 per cent were doubtful, as described earlier, and 62 per cent were negative. Of the 21 knots which were positive for the crowngall organism, four showed a predominance of the characters of wound overgrowth, five showed a mixture of those of wound overgrowth and of crowngall, and 12 showed only the characters of crowngall. Some mixture of the characters of crowngall and wound overgrowth is to be expected, since both types of malformation depend upon injuries for their initiation. As in the preceding year, the efficiency of the technique was tested against known material at frequent intervals. Positive results were obtained from 26 or 27 known crowngalls on inoculated apple trees and naturally infected peach, plum, and cherry. The difference in the percentage of crowngall on the trees examined in 1924–1925 and in 1925–1926 is not surprising when consideration is given to the facts (a) that the material studied in the two seasons came for the most part from different locations, (b) that variation from season to season in the percentages of both wound overgrowth and crowngall is to be expected, and (c) that the small malformations were not included in the studies made in 1925–1926.

A review of the results of the isolation studies in the two seasons show that the technique used gave positive results from 70 of the 73 control trials with known crowngall, and negative results from a high percentage of the enlargements which possessed the typical characters of wound overgrowths. Although much care has been exercised to develop and check the accuracy of the isolation technique, the possibility which is inherent in the negative aspect of the results still remains that further study may possibly reveal the presence of a causal organism in wound overgrowths. However, in view of the facts (a) that Hedgcock (3) reports no isolation studies, (b) that Smith *et al* (25) report only a few isolations from apple which may or may not have been from the type of malformation under consideration and which yielded a considerable percentage of negative results (25, p. 53), (c) that the cultural studies reported herein have consistently failed to reveal the crowngall organism in typical wound overgrowths, although the same technique isolated it consistently from known crowngalls, and (d) that wound overgrowths have been induced or suppressed practically at will under conditions designed to exclude the crowngall organism, as reported on later pages, there seems to be no justification at present for concluding that *Bact. tumefaciens* is the cause of these malformations.

The juncture between the cion and stock appears to be intimately associated with the development of most of the wound overgrowths. An examination of the surface characters of the malformations on two- and three-year-old trees which were grown from piece-root grafts showed that approximately 85 per cent occurred at the union. Of this number about 90 per cent were associated with the lower tip of the cion. This relationship is more clearly observed when sections are made through the trees in such manner as to reveal the original union. Surface and sectional views of such trees may be seen in Plates XXXIX, A, B, C, and XL. In many cases the original wood of both the cion and stock may be observed. Such cases reveal very clearly the tendency for the enlargements to develop about the lower tip of the cion at a place where cambial connection with the root either has been quite poor or has failed entirely. The origin of these knots on the lower cion tip is more easily observed in one-year-old trees. Sectional and surface views of some of these may be seen in Plates XLI and XLVI, B, C, D. Here again, but more clearly than before, it appears that these enlargements have their origin in the lower end of the cion which has not been fitted properly to the root. Sections through one-year-old trees often reveal the original wood of the graft and show how the grafted parts were fitted together. It may be observed in Plate XLI, A, C that the cambium on the lower tip of the cion was not matched against that of the root in such manner that union of these parts could take place.

Callused grafts provide an excellent opportunity to observe the initiation of these enlargements. Such grafts may be seen in Plate XLIV, B. Here it appears that the beginning of knots may follow certain types of misfit in grafting. It is conspicuous that grafts which have the lower cion tip cut and placed in such a manner that the cambium layers of the cion and stock do not closely approach each other are quite likely to develop callus overgrowths. Such a condition is particularly apt to occur when the lower cion tip is too long (Pl. XLIII, C) or when the cion is larger in diameter than the root (Pl. XLIII, F). It may also occur if the lower tip is blunt (Pl. XLIII, D), if it is cut in such a manner that it naturally stands away from the stock when the graft is not tightly wrapped, or if the wrapper rots away too quickly (Pl. XLIII, B). It appears to be important that the wrapper should hold tightly until union between the cion and root becomes well established. Otherwise there seems to be a tendency for a cushion of callus to form under the lower cion tip and to push it away from the stock (Pls. XLI, A to C and XLIV, A, B). In some cases this appears to be serious enough to initiate the development of a knot. Cases in which the cambium of the lower cion tip is cut short of that on the root (Pl. XLIII, A) or in which it projects over very slightly are of less importance to the initiation of wound overgrowths.

Thus, it appears that the development of most of the wound overgrowths may be traced to a beginning in poorly united cions and stocks. Since no causal organism has been found to be associated with the great majority of these enlargements in any stage of their development, it seems necessary to seek other causal factors. The consequences of wounds appear to be, in themselves, sufficient to lead to the production of these overgrowths. A discussion of some of the physiological aspects of the development of wound overgrowths is given on a later page. Meanwhile, a consideration of the fit of a number of commercial grafts is pertinent.

A variety of misfits at the union was found in different samples of commercial apple grafts which were examined. Since the fit at the lower tip of the cion appeared to be particularly important the grafts were classified according to the position of the cambium on this part of the cion relative to that on the stock. Several different classes were arbitrarily chosen. The class "short" (Pl. XLIII, A) included the grafts in which the cambium of the lower cion tip did not come down as far as the cambium on the root. Class "even" (Pl. XLIII, B) included those in which the cambium layers were in close proximity at this critical point. Class "over" included those in which the cambium of the cion tip extended below that of the root by less than 1/16 inch. Class "long" (Pl. XLIII, C) included those in which the cambium of the cion tip extended below that of the root by more than 1/16 inch. Class "blunt" (Pl. XLIII, D) included those in which the lower cion tip had been long, but was cut off at the time the graft was made. In addition to these types a considerable number of grafts were found on which the cambium layers at the lower cion tip were opposite each other, but which were wrapped in such manner that these cambial surfaces were not brought in contact. Such grafts have been included under the additional classification "loose wrap" (Pl. XLIII, B). In grafts where the diameter of the cion was greater than that of the stock, a certain portion of the cambium of the cion obviously could not come in contact with that of the root. Such grafts were given the additional classification "cion large" (Pl. XLIII, F). The results of classifying ten samples of grafts into these different groups may be seen in table 1. Sample No. 1 was received with the notation "well fitted grafts." This was true as applied to the relative diameters of cions and stocks, but not with reference to the fit at the lower cion tip. In view of this notation the question is raised as to whether earlier investigators (Hedgcock, 3, and Melhus and Maney, 8) found comparatively little difference between well fitted and poorly fitted grafts in relation to the development of enlargements because their criterion of fit may have been based more on relative diameters of the stock and cion, and less on the matching of cambium layers at the lower cion tip. From an examination of this table it appears that a sufficient number of misfits is present among the grafts to

TABLE 1.—*Classification of ten samples of commercial apple grafts according to the fit of cion to stock*[a]

Sample number	Total no. examined	Short	Even	Over	Long	Blunt	Loose	Cion large
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	520	20	43	19	17	1	16	1
2	100	8	29	23	14	26	79	4
3	417	12	14	4	16	54	62	15
4	4403	15	15	16	27	27	33	1
5	860	16	30	16	22	16	46	0
6	1041	2	13	5	21	59	0	0
7[b]	84	36	47	17	0	0	0	0
8	134	31	30	20	17	2	0	0
9	205	9	49	21	17	4	0	0
10	214	7	3	14	66	10	0	0
Average		16	27	15	22	20	23.6	2.1

[a] For explanation of classifications used see text.

[b] This sample was sent in as "perfectly fitted grafts."

account for most of the wound overgrowths developed in the field. This subject will be discussed more fully on later pages.

The number of poorly fitted grafts is not surprising when one considers the speed with which they are prepared. In some places the workmen who make the grafts are required to cut and fit at least 3,000 in a ten-hour day, an average of one in 12 seconds. For each graft a slanting cut and a cut for the tongue must be made at the lower end of the cion, and a cross cut at the top. Three similar cuts must be made on the root. After this the cion and a root of the proper diameter must be fitted together. Under these conditions a considerable number of misfits would seem to be inevitable.

Although the lower tip of the cion is the most common location at which wound overgrowths occur, they are sometimes found elsewhere. They may be found extending along the side of the union (Pl. XLVII, D). This is especially apt to occur when the cion is larger than the stock (Pl. XLIII, F) and consequently a considerable portion of the cambium on the cion is unable to make contact with that on the root. It occasionally happens, too, that a knot will be formed at the point of the tongue which extends down from the cion. At times this portion of the cion extends out from the root in such manner that the cambium does not make contact (Pl. XLIII, F, b). Knots have also been found just above the upper tip of the root (Pl. XLVI, B, b). Wound overgrowths seem more apt to occur in this position when a very long slant has been made on the cion and a portion of the cut surface is not covered by the root. In addition to these malformations which appear

to be associated with poor fit of the grafted parts, knots were sometimes found on unions which were first smooth, but later wounded.

Mechanical injuries often appear to initiate the knots which develop on the cion wood of the young apple trees above the unions. Such injuries are commonly made by the careless use of cultivators, hoes, and other tools and by insects. Where wounds remove a portion of the bark and expose the woody part of the stem the results closely parallel those which follow poor fits in grafting. Under favorable environmental conditions callus is formed in a short time and later wound overgrowth develops (Pl. XXXIX, A, B, D). Five to ten per cent of the wound overgrowths on grafted apple trees and nearly all those on budded trees appeared to be initiated by mechanical injuries. During the time a tree stands in the nursery row there is considerable opportunity for injury of underground parts. In the specimen shown in Plate XXXIX, A, B, the diameter of the main axis above the wound overgrowth is considerably greater than that below. This condition suggests that a considerable portion of descending food passed into the roots which originated in the wound overgrowth. Such a situation is not uncommon in wound overgrowths, but has not been observed in cases of known crowngall. Although apparently not so important as the proper fitting of grafted parts, mechanical injuries are a noteworthy factor in the production of wound overgrowths.

Thus far evidence has been presented to show that wound overgrowths are much more numerous on apple trees in the commercial nurseries than crowngall, that they differ from crowngall in various characters, that they appear not to be induced by the crowngall organism, that they develop following poor fits in grafting and mechanical injuries, and that in commercial propagation a sufficient number of poorly made grafts is ordinarily found to account for most of the wound overgrowths which develop in the nursery. A consideration of the experimental production of callus and wound overgrowths follows.

PRODUCTION AND SUPPRESSION OF CALLUS AND WOUND OVERGROWTH

A brief consideration of the development of excess callus and wound overgrowth may be of assistance in the interpretation of results of experiments which deal with their production or suppression. It is commonly accepted that the elaborated foods which are produced by the leaves of a growing apple tree pass down through the stem in a rather straight course with comparatively slow lateral movement. Roberts (21) has made use of this fact in explaining some phases of growth as related to the method of preparing grafts. When this food reaches a place in its downward flow where the direct channel is stopped, and where lateral transfer is difficult, it tends to accumulate. Such a situation, for example, occurs in a cutting or in a

recently planted graft on which the lower cion tip extends beyond the cut on the root (Pl. XLIII, C, a). When this happens under suitable environmental conditions, rapid cell division is stimulated. Ordinarily the exposed surface of the cambium proliferates most rapidly and forms the well known Leyden wedge and a considerable portion of the callus overgrowth. The influences of temperature and, even more particularly, of moisture are of very great importance in determining the extent of callus development. In moist soil or in moist packing material where drying is precluded, a considerable development of soft callus tissue may occur. The surface of this tissue is granular, and growth occurs primarily by the division and enlargement of the surface cells. After some time a phellogen develops, woody deposits occur in the walls of some of the interior cells and a cambium is differentiated which produces the usual tissues on either side. Root primordia may develop, and in some cases the enlargement may be covered with a large number of small roots which give the appearance of one type of hairy root. In some cases the small roots appear to grow together and give the overgrowth the aspect of a ball of knotted string. Wound overgrowths have been observed to reach considerable size on some plants and to continue their development for several years. Wounds above ground on apple trees form very little soft callus tissue because of the drying influence of the atmosphere. Such wounds are covered slowly by the overgrowth edge as described by Sorauer (28). Injuries such as those from cultivators, hoes, hooks, and insects may serve to stop the downward passage of elaborated food and to lead to developments similar to those which follow a poor fit in grafting. Such developments are normal processes of the plants when suitable conditions are provided.

The lower tip of the cion is a particularly important place in relation to the development of callus. This has already been observed in relation to the very common position of the knot on the nursery trees, and to the position of the callus on the lower tip of the cion. This relationship was still more apparent when the cions were cut to a feather edge and permitted to callus without having a root attached. Two hundred cions were cut in this manner, placed in new moist sawdust such as is used for packing grafts, and stored at temperatures varying from 10° to 15° C. for two months. At the end of that time all but two had developed active callus around the cut surface of the cambium. By far the greatest development had occurred at the sharp lower end (Pl. XLIV, A). At this place the cell proliferation had been so great that a considerable mass of callus tissue had developed, which in most cases was as large in diameter as the original cion wood. Cultural examinations of these enlargements were negative for the crowngall organism. It seems probable that the food as it descended the twig to the cut made a slow lateral movement along an obtuse angle to the lower tip where

it accumulated and contributed to the development of callus. In a well fitted graft (Pl. XLIV, C) such an accumulation occurred in a much smaller degree because the food material passed through the uniting cambium layers into the root. However, as may be seen in Plates XLIII, G, and XLIV, B, this formation of callus is common where the root and cion are not properly united.

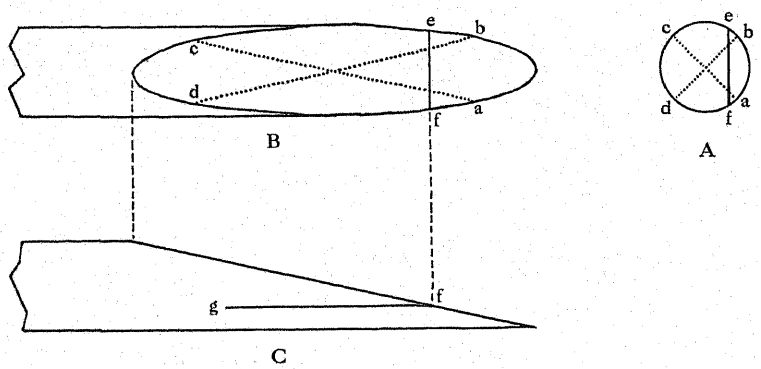

FIG. 2.—Diagram of the lower part of the cion of a commercial piece-root apple graft.

The fit of the lower tip of the cion is important also because there is less cambial surface exposed and therefore less opportunity for union on this sector of the graft than on the side. This is shown in figure 2, which was prepared from a representative commercial apple graft. Here A represents an end view, B a front view, and C a side view of the slanting cut on a cion. Lines *ef* and *fg*, respectively, represent the slit in the cion tip across the front and down the side, into which the tongue of the stock is to be inserted. Suppose that the cion should be quartered by planes which coincide with the lines *ac* and *bd* so that the extreme tip is included in the quarter bound on the outside of the curve *ab*. Now, since the union between the stock and cion takes place almost completely through the callus initiated by cambium, the sector bounded on the outside by the curve *ab* has only the area about this curve in which to unite. However, the sector of the cion bounded on the outside by the curve *ad* has not only the area about *ad* but also that about *fg* and *gf* in which to unite. It should be remembered that in the actual graft the tongue of the root is inserted in the cut represented by the line *fg* (compare with Pl. XLIII). Actual measurements show that the distance *ab* is less than one third of $ad + fg + gf$. So far as cambial surface is concerned, the chances for union are just as small for the sector bounded on the outside by the curve *cd* as for that bounded by *ab*. However, in actual practice the stock is usually cut so that any callus which develops

about the cambium represented by *cd* soon comes in contact with callus on the stock. At the same time, as the metabolic products come down the stem in the quarter bounded on the outside by the curve *cd*, they may, by slight divergence along an obtuse angle, continue their passage. Such translocation would be much more difficult if not impossible in the quarter bounded on the outside by the curve *ab*.

The influences of temperature and moisture on the development of callus on apple grafts were studied. The results conformed so closely with the well established information on this subject that the details of these experiments are omitted. It was found that within certain limits callus development increased with higher temperatures and moistures and decreased with lower temperatures and moistures. Grafts which had formed comparatively little callus at the time of planting grew better than those which had produced excess callus.

The relation between excess callus development and the fit of grafts was tested in preliminary experiments in the greenhouse. Approximately a hundred commercial grafts taken at random were sorted according to the fit of the lower cion tip and planted in pots. The different classes into which they were sorted have been described. After three months the grafts were all dug and further classified according to the amount of callus that had developed. The results appear in table 2. When six of these callus forma-

TABLE 2.—*The relation of fit of cion and stock to the development of overgrowths on commercial Wealthy grafts grown in the greenhouse for three months*

Fit of cion tip[a]	Type of wrap	Dead	Size of callus formed on cion			Total
			None or less than ¼ diameter of cion	¼ to ¾ diameter of cion	Over ¾ diameter of cion	
		No.	No.	No.	No.	No.
Short	Tight	.....	2	.....	.....	2
	Loose	1	3	2	.....	6
Even	Tight	.....	2	1	.....	3
	Loose	5	10	11	.....	26
Over	Tight	.....	1	.....	.....	1
	Loose	.....	1[b]	9	2	12
Long	Tight	1	.....	10	1	12
	Loose	3	.....	6	2	11
Blunt	Tight	1	.....	2	.....	3
	Loose	4	3	11	4	22
Total		15	22	52	9	98

[a] For explanation of classification used see text.

[b] Lower extremity of cion tip dead.

tions were cultured, all were negative for the crowngall organism. These data indicate that grafts which have the lower cion tip cut in such manner that its cambium fails to make contact with that of the root are more likely than well fitted grafts to produce excess callus. Furthermore, the larger amount of callus which developed on properly fitted but loosely wrapped grafts emphasizes the value of tight wrapping. This seems necessary to prevent the lower cion tip from being pushed away from the stock by the development of a cushion of callus tissue.

The importance of the fit of the cion was further tested in preliminary experiments in which grafts were made with care to secure types of good fit and of poor fit at the lower cion tip. At the same time precautions were taken to exclude *Bact. tumefaciens*. The roots were thoroughly washed and the cion wood and roots were dipped in mercuric cyanide, 1–1,000. The knives were dipped before each cut in mercuric chloride, 1–1,000. These antiseptics were employed because Reimer (14) had found them to be very effective in his work with fire blight. The grafting was done in a transfer chamber to minimize chances for contamination. The grafts were wrapped with ordinary unwaxed No. 18 cotton thread which had been autoclaved. The hands of the operators were dipped in mercuric chloride, 1–1,000. After the grafts were made they were planted in soil which had been placed in ordinary greenhouse flats and autoclaved on each of three successive days for five hours at 15 pounds pressure. The pots used were new and had been dipped in mercuric cyanide, 1–1,000. After the grafts were planted they were placed in a greenhouse where there were no plants with crowngall. The experiment was begun in February, 1925. Twenty of the grafts were accurately fitted. After two months all were alive and vigorous, and none of them had developed excess callus (Pl. XLIV, C). Fifty grafts were made with the lower cion tips long. After two months four were dead, four had decayed lower cion tips, and 42 had well developed masses of excess callus (Pl. XLIV, B), with the characteristic granular appearance. Cultural examinations were made of the interior parts of five of these enlargements, but most of them were sterile and all were negative for the crowngall organism. These callus formations appear to be identical with those which are found on commercial grafts (Pl. XLIII, G). These experiments strongly indicate that excess callus formations typical of those which occur so commonly about the unions of grafted apple trees in the nursery may be developed following misfits in grafting, without the intervention of the crowngall organism.

Field experiments were performed in 1923 in which precautions were taken to exclude the crowngall organism. The roots and cion wood were dipped in mercuric cyanide, 1–1,000, before grafting. Before each cut the knives were treated with mercuric chloride, 1–1,000. Grafts were cut and

fitted as in ordinary commercial propagation and wrapped with untreated cotton thread. They were then placed in clean sand until the cions and stocks were well united by callus. They were planted at Madison, Wisconsin, in soil which had been steamed as described by Johnson (4) for tobacco beds. A railing of boards was placed about the treated soil so as to prevent surface water from running into the plot. Of the 74 trees, which were harvested in the fall after a season's growth, diagnosis based on external characters revealed that 51 were smooth, 16 showed wound overgrowth at the union (Pl. XLV, E), and 7 showed hairy root (Pl. XLV, A, B, C). None showed the symptoms of crowngall. Eight of the trees showing malformations were subjected to bacteriological examination. All were negative for the crowngall organism. The wound overgrowths in this case were quite hard and bore a considerable number of root primordia.

In 1924 a series similar to the above, except that the grafts were not made under aseptic conditions and were planted in unsteamed soil, yielded the following results, based on external characters after one season of growth. Of 677 trees which were harvested 446 were smooth, 225 showed wound overgrowth at the union, five showed hairy root at the union and one showed crowngall on the cion above the union. It is worthy of note that in each of these last two experiments approximately one-third of the total number of trees harvested showed malformations at the union. It is quite common for the nurserymen to find that a third or more of their Wealthy trees bear root knot. These experiments indicate that except for one specimen the crowngall organism was not a factor in the development of the malformations found on these trees.

Similar experiments were performed in the season of 1925. This year the roots but not the cions were dipped in mercuric cyanide, 1–1,000, before grafting. After callusing in moist sawdust they were planted in soil which had been steamed and enclosed by boards as in the earlier experiment. These grafts had been prepared with a considerable variety of fits and misfits at various parts of the union. A detailed account of the relations of the several types of fits to wound overgrowth formation will be given on a later page. Only those trees which were more than a foot high were considered when results were taken at the end of the first growing season. Diagnosis based on external characters showed that, of the 1,008 trees harvested, 455 were smooth, 271 bore small wound overgrowths at the union (Pl. XLVI, D), 280 bore large wound overgrowths at the union (Pl. XLVI, C), and none bore crowngalls. Uniformly negative results from cultural examinations of ten of these malformations conform with the diagnoses made on the basis of external characters.

Small field plantings of commercial apple grafts were made in unsteamed soil. Of the 527 trees harvested at the end of one growing season

and diagnosed on the basis of external characters, 282 were smooth, 126 bore small wound overgrowths at the union, 115 bore large wound overgrowths at the union, and four bore bacterial galls, one on the cion and three at the union. The diagnosis was checked by cultural examinations of eight representative wound overgrowths, all of which were negative for the crowngall organism, and two crowngalls, both of which were positive. The results of these experiments corroborate those of the experiments of the two previous years, indicating that *Bact. tumefaciens* played only a minor rôle in the formation of the knots on the trees examined.

Excess callus followed by wound overgrowth has been induced on older trees by wounding under conditions in which precautions were taken to exclude the crowngall organism. On March 12, 1925, 29 three-year-old Jonathan trees were thoroughly washed and then dipped in mercuric cyanide, 1–1,000. After this a slanting cut which extended approximately half way through the stem was made with a disinfected knife, sufficiently close to the union that the injury would be under the surface of the soil when the tree was planted. The cut slanted toward the top of the tree at approximately a 45° angle. In order to prevent direct healing a thin strip of inert substance was inserted in the cut. With seven trees mica was employed, while with the remaining 22 a small piece of medical adhesive plaster was placed in each cut. The trees were then planted in new pots which had been dipped in mercuric cyanide, 1–1,000. The soil used had been placed in small flats and autoclaved for five hours at 15 pounds pressure on each of three successive days. The potted trees were placed in a greenhouse where no other plants were growing. Six of these trees were examined May 19. Well developed callus (Pl. XLVI, A) was observed at the injury in four cases, while in two cases wound overgrowths had developed. Isolation studies made on these enlargements were negative for the crowngall organism. The remainder of the trees were examined August 26, 1925. With the exception of two which died all showed wound overgrowth (Pl. XLV, D). In two cases where a bud was close to each injury hairy root developed. Although precautions had been taken to exclude *Bact. tumefaciens* from these injuries, cultural examinations of three representative enlargements were made. They were negative for the crowngall organism. The characters of these enlargements were the same as those given previously for the wound overgrowth. In view of the obvious difficulties attendant upon growing apple grafts under aseptic conditions, no attempt was made in these and previous experiments to exclude all microorganisms throughout the period of the test. However, it is considered that the technique used furnished sufficient safeguards against the presence of the crowngall organism to make the possibility of its intervention exceedingly remote.

Similar results have been secured in the field following different injuries. One-year-old trees in four lots of 12 each were injured on the cion above the union but below the ground line. The first lot was bruised with a hammer, the second was given a series of needle punctures, the third was given a deep cut which slanted upward, and the fourth had the same treatment as the third except that adhesive tape was inserted in the cut. The trees were planted and left for an entire growing season. They were in rather poor condition at the time of planting. This factor, rather than the injury given them, is probably responsible for the large number which died. The first lot, which was bruised, produced two smooth trees, two which bore hairy root, and eight which had wound overgrowths. The second lot, which received punctures, produced five trees with small and one with large wound overgrowths. The other six died. The third lot, which was cut, produced five small and one large wound overgrowths. The other six died. The fourth lot, which had tape placed in the cut, produced five medium sized and two large wound overgrowths. The other five died. Similar experiments were tried on three-year-old trees. Twenty-five trees were either bruised as in the first lot above, or cut and treated as in the fourth lot. None of them died. In one season 19 of them produced wound overgrowths which were of such size that they would have been rejected during nursery inspection. Cultural examination of representative enlargements from these experiments were negative for the crowngall organism. These experiments give further evidence that wound overgrowths are developed without the intervention of the crowngall organism.

All the experiments and observations which the writers have made point consistently to the conclusion that, except for a comparatively small percentage which usually had distinctive characters, the enlargements which they studied on apple nursery stock were not caused by the crowngall organism. On the other hand the characters of these malformations made them appear to be identical with wound overgrowths produced by poor grafting and wounding. Consequently their control appears to be most readily approached by modifications of horticultural practices, rather than by efforts to prevent infection by the crowngall organism.

The relation of the fit of the graft to the development of wound overgrowth in commercial practice was studied by sorting commercial grafts obtained as representative of those prepared by certain nurseries into the several classes described earlier and planting them in the field. Approximately half the grafts were planted in steamed soil. At the end of the first growing season an examination based on external characters showed that crowngall had developed on less than one per cent of the trees in unsteamed soil and on none in steamed soil. Cultural examinations of 10 wound overgrowths were negative for the crowngall organism. In view of these facts

the data on crowngall are omitted and those concerning the development of wound overgrowths on both of these plots are merged (table 3). As the trees were dug they were classified as to whether they were smooth or bore wound overgrowths. These enlargements were further divided into groups called "small" and "large" according to the standards shown in Plate XLVI, C, D. The results taken on these trees, which were grown at Madison, Wisconsin, in the season of 1925, are given in table 3. Here it appears that

TABLE 3.—*Correlation between the fit of the lower tip of the cion to the root of commercial Wealthy apple grafts and the development of wound overgrowths during the first season*[a]

Fit at lower tip of cion	Grafts planted	Stand over 1 ft. high[b]	Smooth[c]	Small knot	Large knot
	No.	Per cent	Per cent	Per cent	Per cent
Short	149	59	65	23	12
Even	274	64	79	13	8
Over	207	61	52	29	19
Long	466	54	24	38	38
Blunt	558	61	47	32	21
Average		60	53	27	20

[a] The grafts were hand wrapped with waxed No. 18 cotton thread. The figures include both tightly and loosely wrapped unions. No grafts with cions larger than the stocks were employed. For explanation of classifications used see text.

[b] Six per cent of the trees harvested had small callus formations on the cions, probably due to mechanical injuries.

[c] Eight per cent had wound overgrowths at some location other than the lower tip of the cion. On one per cent the lower tip of the cion was dead.

the harvest from grafts with cion tips "even" contained a smaller number of wound overgrowths than that from any other class. In this class with well fitted unions a still lower percentage might have been expected had all the grafts been tightly wrapped. As has been mentioned earlier the cion tips which are not securely held in place often may be pushed away from the root as the callus develops, even when they are cut to the proper length. The group of grafts with long cion tips (Pl. XLIII, C) yielded the highest percentage of wound overgrowths, while the other groups produced intermediate percentages.

It is to be observed that, although approximately three-fourths of the grafts with long lower cion tips produced trees with enlargements, about one-fourth produced smooth trees. Although much still remains to be learned about the formation of wound overgrowths several rather obvious factors which might account for these smooth trees should be mentioned. (a) It was quite apparent on some of these trees that wound overgrowths did not

develop at the lower tip of the cion because of the death of tissues in this region. However, this would account for only a very small portion of the trees which did not bear enlargements, except in certain cases where the grafts were treated with antiseptics. In one lot of grafts which were prepared with long cion tips and wrapped with unwaxed cotton thread, dipped in a commercial preparation of hydroxymercurichlorophenol, 1–400, and planted in pots in the greenhouse for two months, the lower tips of 50 per cent of the cions were killed. Of the remaining 50 per cent a large majority developed typical excess callus formations at the lower cion tips. The stand was not reduced by this treatment. On control grafts in this experiment death of the tissues of the lower cion tips occurred only in occasional instances. (b) In case good union occurred on both sides of the cion tip the food was apparently transferred slightly to one side or the other and so failed to accumulate in the cion tip. (c) It has been observed sometimes that the callus on the lower cion tip may send out roots very early in its development. In such cases the roots may carry off the food material, and thus prevent its accumulation in the lower cion tip (Pl. XLVII, B). Unfortunately, with the exception of grafts treated with certain antiseptics, these and other influences did not seem to operate on the Wealthy variety to make smooth trees from more than approximately a quarter of the grafts that had long lower cion tips.

The importance of the lower cion tip in production of wound overgrowths was further shown in an experiment in which the wedge graft was employed.The lower end of the cion was sharpened to form a wedge and inserted into a split in the upper end of the stock. In each case the cion was equal to or smaller than the root in diameter. The grafts were wrapped with waxed string and planted in steamed soil for the growing season of 1925. Of 200 grafts planted, 105 were over a foot high when harvested. Only 7 of these showed any wound overgrowth. No crowngall was found. This experiment and those described on earlier pages show that the fitting of the grafted parts, particularly at the lower tip of the cion, is important in relation to the occurrence of wound overgrowths.

The influence of the diameter of the cion relative to that of the stock was tested with a limited number of grafts. The grafts in this experiment all had a difference in diameter between the cion and stock of at least one-sixteenth of an inch. They were planted in the spring of 1925 at Madison, Wisconsin, in soil which had been steamed out of doors by the method used in earlier experiments, and were harvested at the end of the first growing season. The results are shown in table 4. For comparison the average from table 3 is shown as a commercial lot. Here it may be noted that the grafts in which the cion was smaller than the stock (Pl. XLIII, E) produced trees with approximately the same number of wound overgrowths as the

commercial lot. However, the grafts in which the cions were larger in diameter than the stocks (Pl. XLIII, F) produced a much higher percentage of wound overgrowths. This type of fit of necessity left some cambium on the cion exposed in such a manner that union with the cambium on the root was difficult. These results indicate strongly the positive correlation between the fit in which the cion is larger than the stock and the development of wound overgrowth.

TABLE 4.—*Correlation between the relative diameter of cion and stock of commercial Wealthy apple grafts and the development of wound overgrowths during the first season*[a]

Classes	Grafts planted	Stand over 1 ft. high	Smooth	Small knot	Large knot
	No.	Per cent	Per cent	Per cent	Per cent
Commercial[b]	1654	60	53	27	20
Cion larger than stock	100	56	9	27	64
Cion smaller than stock	100	50	46	32	22

[a] These grafts were wrapped with waxed thread. For explanation of classifications used see text.

[b] A sample lot of grafts obtained from a nursery.

The influence of several types of wrapping on the development of wound overgrowths was also studied. The different wrappers were placed on commercial grafts made by the same operators who prepared the grafts which were sorted for the planting referred to in table 3. For this reason the average from table 3 is entered for comparison with the results of this experiment. As described in the preceding paragraph the grafts were planted in steamed soil and left through the growing season of 1925. The results at the end of one growing season are given in table 5. Here it may be seen that with loose wrapping (Pl. XLIII, B) the percentage of wound overgrowth was increased. With cloth, which provided a more complete covering than did the string, the amount of wound overgrowth was decreased from the average. However, it should be noted here that untreated cloth rots more rapidly in the soil than does waxed string. The percentage of wound overgrowth was still more decreased with waxed paper, which did not rot so quickly. With the strongest and most persistent wrapper employed, common medical "ZO" or "Zinc oxide" adhesive plaster, there was the smallest percentage of wound overgrowth. This wrapper held the grafted parts very firmly together, prevented the incorporation of soil particles in the callus and opposed the development of excess callus by a firm

binding action which lasted over the most critical period for the initiation of wound overgrowths. However, it rotted (table 6) before any evident girdling effect was induced (Pl. XLVII, A). The diagnosis of these wound overgrowths was based on external characters and checked by cultural examinations of four representative trees, all of which were negative for the crowngall organism. It appears that a tightly applied wrapper which holds the grafted parts securely together without rotting too quickly or lasting long enough to induce girdling is an important factor in preventing the development of wound overgrowths.

TABLE 5.—*Correlation between the kind of wrapping at the union of commercial Wealthy apple grafts and the development of wound overgrowths during the first season*

Type of wrapper	Grafts planted	Stand over 1 ft. high	Smooth	Small knot	Large knot
	No.	Per cent	Per cent	Per cent	Per cent
Waxed string.....	1654	60	53	27	20
Waxed string (loose wrap at lower end).....	312	52	36	30	34
Adhesive plaster	100	47[a]	94	2	4
Waxed paper.....	290	73	72	17	11
Cloth wrap.....	100	57[a]	67	21	12

[a] Planted in comparatively poor soil. In other experiments, grafts which were bound with this wrapper and planted in better soil equalled or surpassed those in commercial plantings.

The efficiency of the adhesive plaster used was apparently not due to any germicidal action. Crowngall bacteria from an agar slant were placed on the adhesive surface of a strip of plaster like that used in wrapping the experimental grafts. The plaster was then placed in a petri dish with some strips of moist filter paper to prevent desiccation. At the end of seven days the bacteria on the adhesive surface were still viable. It seems that the zinc oxide is placed in this plaster to prevent irritation of the human skin, and that nothing in the preparation is intended to exert a bactericidal action.

The durability of several of the more common or promising wrapping materials was tested by winding them on sticks and burying them for different periods in ordinary soil with a moisture content suitable for growing plants. At the end of the experiment the tensile strength of the wrappers was measured with a device set up after consultation with M. E. Dunlap of the United States Forest Products Laboratory. The upper end of the material to be tested was fastened in a clamp, and a container was sus-

pended from the lower end. An evenly applied load of sand was poured into the container through a funnel. The weight necessary to break the material was determined. The results, which are the averages of 10 measurements on each lot of material, are given in table 6. It appears that ordinary cotton thread, cloth, and even raffia rot very rapidly. Waxed string and adhesive plaster retain considerable strength for several weeks. Hydroxymercurichlorophenol seems to have exerted a preservative action on both cloth and raffia. It seems, therefore, that, although various chemical substances have proven of very doubtful value in preventing infection by the crowngall organism, some of them may have a distinct value in preserving wrappers and thus indirectly inhibiting the development of wound overgrowths. Further experiments in this direction appear to have considerable promise.

From the several experiments which have been reported above, it appears that excess callus and wound overgrowths may be induced where precautions are taken to exclude the crowngall organism and that, within limits, they may be initiated or suppressed by modifications in cultural practices. Control of these malformations may be accomplished without the use of anti-

TABLE 6.—*Measurements in kilograms made to determine the average tensile strength of one inch lengths of several apple graft wrappers after different periods of time in soil*

Wrapper	Tensile strength after stated intervals:			
	9 weeks	6 weeks	3 weeks	New material
	kg.	kg.	kg.	kg.
No. 18 cotton thread	Disintegrated	Disintegrated	Trace	0.7
No. 18 cotton thread waxed	Trace	0.5	0.7	0.5[a]
Adhesive plaster ½ in. wrapped without overlapping	Trace	0.8	4.8	10.1
Adhesive plaster ½ in. wrapped with overlapping	1.5	4.3	5.9	10.1
Unbleached muslin ½ in. strips	Disintegrated	Disintegrated	Trace	4.5
Unbleached muslin ½ in. strips treated[b]	Disintegrated	Trace	4.6[c]	.....
Raffia	Trace	Trace	0.5	8.4
Raffia treated[b]	Trace	1.0	2.5	.....

[a] When the thread was first waxed the fibers appeared to slip by one another more easily than in untreated thread. This reduced the strength somewhat until the wax became set.

[b] The treatment with hydroxymercurichlorophenol was as follows: The material left nine weeks was dipped in the solution 5 minutes, dried, and dipped again for 5 minutes. The other material was all given two treatments of approximately 6 hours each.

[c] The cloth strips were a trifle wider than ½ inch.

septics, although the latter appear to have important potentialities in the preservation of wrappers. The following practices seem to be among the more promising for the control of wound overgrowths: (a) using cions equal to or smaller in diameter than the corresponding stocks, (b) proper fitting of grafts, especially at the lower tip of the cion, (c) wrapping tightly, especially at the lower tip of the cion, with some material which remains firm through the critical period of callus formation and then rots before it induces girdling, (d) avoidance of excessive callus formation in storage, and (e) prevention of injuries during cultivation.

RELATIVE PREVALENCE OF CROWNGALL AND WOUND OVERGROWTH

When it was found that crowngall and wound overgrowth are due to different causes and may be distinguished with considerable accuracy, an effort was made to determine the relative importance of these malformations in different regions.

A survey was made for crowngall and similar abnormalities among some of the apple nurseries of the North-Central and the Northeastern United States. Representative nurseries which were growing apple trees were visited and the trees examined as they were dug. Where it was impracticable to examine very large numbers the percentages were secured from counts on several hundred trees taken at random. The results, which are summarized in table 7, show that crowngall is very widely distributed in the area surveyed but that it occurred on a very small percentage of apple trees in most nurseries. In the nurseries surveyed, wound overgrowths were generally of very considerable importance on grafted apple trees, but were of comparatively little consequence on budded apple trees. This difference appeared to be due primarily to the wound overgrowths which developed as a result of misfits in grafting. Practical control of wound overgrowths on grafted apple trees was observed in three commercial nurseries in Connecticut, Pennsylvania, and West Virginia (table 7). In each case the nurserymen explained their results on the basis of care in the preparation and handling of the grafts. All used waxed string wrapping and employed no antiseptic. Cultural examination of representative specimens from a number of the nurseries surveyed gave results which confirmed the field diagnoses. The results of this survey agree with those from the other lines of work reported in this paper in showing that, under the range of conditions covered by these studies, the malformations encountered on the underground parts of apple nursery stock were predominantly wound overgrowths rather than crowngall.

TABLE 7.—*Summary of data from survey for crowngall, wound overgrowth, and similar malformations on certain types of nursery stock, 1925*

State	Nursery	Kind of stock	Propagation	Wound overgrowth	Hairy root (all types)	Crown-gall	Woolly aphis
				Per cent	Per cent	Per cent	Per cent
Conn.	A	Apple	Budded	1	1	½	½
		do.	Grafted	2[a]	1	½	½
		Peach	Budded	Trace	0	9	.....
Ind.	A	Apple	do.	Trace	2	Trace	5
		Peach	do.	0	0	Trace	.....
Iowa	A	Apple	Grafted	38	[b]	Trace	.....
		do.	Budded	2	[b]	Trace	.....
	B	do.	Grafted	25	[b]	Trace	.....
		Cherry	Budded	.....	.....	Trace	.....
		Raspberry	.....	.....	.....	Trace	.....
Md.	A	Apple	Budded	8	12	Trace	Trace
		Peach	do.	0	0	0	.....
Mich.	A	Apple	do.	Trace	2	Trace	Trace
	B	do.	do.	Trace	Trace	Trace	25[c]
		Pear	do.	Trace	Trace	Trace	80[c]
	C	Apple	do.	2	8	1	.....
Minn.	A	do.	Grafted	38	4	2	.....
Mo.	A	do.	do.	40	[b]	Trace	.....
		Cherry	Budded	.....	.....	Trace	.....
		Raspberry	.....	.....	.....	3	.....
Neb.	A	Apple	Grafted	33	[b]	2	.....
		Raspberry	.....	.....	.....	5	.....
N. Y.	A	Apple	Budded	½	2	Trace	3
	B	do.	do.	1	13	Trace	1
	C	do.	do.	½	7	½	3
		do.	Seedling	Trace	40[d]	Trace	5
Penn.	A	do	Budded	Trace	2	Trace	1
		do.	Grafted	3[a]	2	Trace	1
		Peach	Budded	0	0	Trace	0
West Va.	A	Apple	Grafted	10[a]	3	Trace	6
		do.	Budded	2	3	Trace	6
Wis.	A	do	Grafted	28	[b]	Trace	.....
	B	do.	do.	24	[b]	Trace	.....
		Raspberry	.....	.....	.....	1	.....

[a] Special care was taken in the preparation of the grafts from which these trees grew.

[b] Hairy root is included in percentage under wound overgrowth.

[c] The diagnosis is doubtful. These were large overgrowths which did not resemble those usually diagnosed as wound overgrowths, bacterial gall, or woolly aphis injury. Because woolly aphis appeared commonly associated with these enlargements, and cultural studies were negative for crowngall, they have been provisionally placed in this column.

[d] These were budded stock on which the bud died. The hairy root in these cases was practically all of the genetic type.

DISCUSSION

Although the results reported in this paper are in general agreement with those of other workers on this subject, they appear to require some important differences in interpretation. These center largely about the differentiation of wound overgrowths from the crowngalls induced by *Bact. tumefaciens*. Earlier workers appear not to have recognized the nature or the importance of these wound overgrowths on apple nursery stock. Consequently, work with two similar types of abnormalities induced by different causes unavoidably led to much confusion in the interpretation of results.

The bacterial nature of crowngall was established by Smith and his coworkers (24, 25, 26), who showed the causal relationship of *Bact. tumefaciens* to crowngalls on a large number of widely different species of plants. Among many other malformations, they studied those on apple trees, to which, however, they devoted relatively limited attention. The present writers have found a record in this work of cultural examination of only 17 root knots on apple, four of which were "soft" and 13 "hard." Positive results are recorded from all the "soft galls" and four of the "hard galls," and negative results from the remaining nine "hard galls." The description of this material does not enable one to determine how many of the "hard galls" may have been wound overgrowths. In this connection, it is pertinent to recall that wound overgrowth and crowngall sometimes merge in one malformation. When these studies were made, the isolation of the crowngall organism was considered to be more difficult than it is at present. Consequently, at that time the positive results were apparently considered to be sufficient basis for the conclusion that *Bact. tumefaciens* was the cause of all the 17 apple root knots cultured.

With the exception of failure to distinguish between crowngall, excess callus, and wound overgrowth, the results of Hedgcock (2, 3) are in many respects in very close accord with those reported herein. He recognized the importance of the fit of cion and stock, the relation of callus to the subsequent development of enlargements, the failure of many antiseptics to prevent the development of malformations, the value of good wrapping, and many other points for which the reader is referred to his papers. Unfortunately, however, so far as the present writers are aware no adequate bacteriological study of Hedgcock's material was made. Consequently, in his later work he appears to have accepted the prevailing conception that *Bact. tumefaciens* was the cause of the enlargements with which he dealt. With the wide acceptance of the parasitic theory of the cause of these malformations, incentive to careful fitting of grafts appears to have been lessened. Many of the nurserymen have increased the speed in the prepara-

tion of grafts, with the expectation of destroying a considerable percentage of the trees because of crowngall infection.

Interpreting their results in accordance with the prevailing conception that they were dealing with a single disease caused by *Bact. tumefaciens*, Melhus and Maney (8) reported reduction in the occurrence of malformations on apple nursery trees, together with some reduction in the stand, following treatments of grafts with Bordeaux mixture. Similarly, Sherbakoff (23) suggested that in some cases a reduction in the percentage of malformations might follow applications of sulphur to the soil in the nursery rows at the time the grafts were planted.

More recently a revision of the earlier inclusive conception of crowngall on apple trees has been in progress. Brown (1), who was co-author with Smith and Townsend in earlier studies on crowngall, has shown through bacteriological studies that many of the aerial knots earlier attributed to the crowngall organism are not induced by this parasite. The present writers have made cultural examinations of 10 such knots, which were secured from West Virginia and New York, and have obtained like results. Swingle (30) has suggested that, since the knots which Brown cultured were apparently non-parasitic, they might be the result of an inherent tendency of the tree. He has listed many varieties of apple according to the prevalence of these developments. The present writers (17, 18) have reported on differences between crowngall and wound overgrowth, with suggestions concerning methods for the control of wound overgrowth. A detailed account of this work is given in the present paper. Recent reports by Melhus (9, 10) and Muncie (12) corroborate these results with reference to the etiology of wound overgrowth. Furthermore, Muncie (11) has reported that certain types of hairy root of apple are noninfectious.

Wormald and Grubb (32) have reported that galls on young apple trees may be induced by various mechanical and chemical agencies as well as by *Bact. tumefaciens*. They state that crowngall may be controlled by care in handling the stocks and by using grafting wax or Stockholm tar.

Waite and Siegler (31) report control of crowngall of grafted apple nursery stock by means of dippings in a solution of hydroxymercurichlorophenol before planting. These investigators appear to adhere to the earlier conception of crowngall, which included the malformations described herein as wound overgrowths, and report no cultural examination of their material. If further study should reveal that they were dealing chiefly with wound overgrowths rather than crowngall, their data on control would agree very closely with the results of the present writers.

Oppenheimer (13) has reported that dipping of apple and pear trees in a solution of hydroxymercurichlorophenol was ineffective against crowngall

infection, but that puddling the trees in a suitable mixture of this compound, soil, and water gave control. It appears that this writer also employs the term crowngall according to the earlier conception, without distinguishing between the bacterial disease and wound overgrowth. In some of his work, however, he used cultures of the crowngall organism.

The segregation of wound overgrowth from crowngall of apple nursery trees appears to clarify numerous perplexing questions, such as: (a) why treatments with certain antiseptics have failed to give control, (b) why certain types of malformations disappeared, (c) why some of the most vigorous trees in the nursery bore large knots and seemed to suffer no ill effects, (d) why reports of injury due to knots in the orchard were very conflicting, (e) why changes to new soil failed to reduce the prevalence of the malformations, (f) why partial control attended the use of certain wrappers and antiseptics, (g) why the occurrence of malformations was minimized by the practice of budding, (h) why cion wood from trees bearing root knots produced no higher percentage of knots than that from smooth trees, and (i) why the majority of the malformations on apple are so different in appearance from crowngall on apple, peach, and raspberry.

The studies herein reported open the way for a new approach to the problem of controlling malformations on grafted apple nursery stock. Since we are concerned primarily with wound overgrowths, most of which are initiated by misfits in grafting or by wounds in cultivation, control may be greatly furthered by improvement in horticultural practices. While much work remains to be done in the refinement of these practices, it has been shown experimentally and demonstrated independently in certain nurseries that the occurrence of wound overgrowths may be greatly reduced by making well fitted grafts, wrapping them tightly with a suitable material, callusing under favorable conditions, and avoiding wounding in cultivation.

SUMMARY AND CONCLUSIONS

1. Crowngall infection of young apple trees was readily induced when *Bact. tumefaciens* was inoculated into wounds of various types. In several experiments, freshly produced callus on apple grafts and young trees failed to serve as an open infection court for this parasite.

2. Crowngall of apple has definite characters which are similar to those of crowngall of peach and raspberry, but are different from those of wound overgrowths on apple, as described herein. Cultural studies have demonstrated that *Bact. tumefaciens* may easily and consistently be isolated from crowngalls of apple in their various stages of development. Crowngalls are commonly comparatively soft, but may at times be hard. The term "hard gall" is not distinctive.

3. Crowngalls are subject to decay by microorganisms. After rotting the galls, these organisms frequently attack the cortex of the young trees, which may be girdled and killed. Crowngalls may overwinter in a sound condition. *Bact. tumefaciens* has been isolated without difficulty from overwintered crowngalls of apple and of raspberry.

4. In the present writers' experiments, many common antiseptics, including certain commercial preparations of hydroxymercurichlorophenol, have proven ineffective in preventing infection of tomato by *Bact. tumefaciens.*

5. Standardized cultural studies of malformations on 407 apple trees taken from several thousand received as "crowngall" rejects from 22 nurseries in 14 states failed to reveal the presence of *Bact. tumefaciens* or any organism closely similar to it in 86 per cent of the trees examined. The same cultural technique applied to typical crowngall on apple, peach, cherry, and plum isolated *Bact. tumefaciens* from 70 of 73 specimens examined. The malformations on the rejected stock which failed to yield the crowngall parasite possessed certain characters which were different from those of crowngall. Because of the relationship of wounds to their initiation and development, they are called wound overgrowths.

6. Wound overgrowths are shown to occur commonly on young apple trees following certain injuries. On grafted apple nursery stock, most such overgrowths develop incidentally to the grafting wounds. They are most abundant, therefore, about the union of cion and stock. They occur most frequently as an overgrowth of the lower tip of the cion. A small number of wound overgrowths on grafted stock and practically all those on budded stock follow injuries induced in cultivation.

7. Differential characters of crowngalls and wound overgrowths are discussed.

8. Examinations of representative lots of commercial apple grafts revealed a sufficient number of misfits to account for most of the wound overgrowths which develop in the nurseries.

9. Under experimental conditions in which precautions were taken to exclude the crowngall organism, wound overgrowths typical of those found in commercial practice have been developed readily from poorly fitted grafts or following wounds. In the same experiments, well-fitted grafts developed smooth unions.

10. Wound overgrowths have been controlled in experimental plots without the use of antiseptics. Important factors in this control were: (a) avoiding the use of cions of greater diameter than the stocks to which they were fitted, (b) proper fit at the lower cion tip, (c) tight wrapping with a material which formed a sufficiently strong bandage about the union and

maintained its strength throughout the critical period for callusing, but rotted before exercising an injurious girdling effect, (d) prevention of excessive callus development in storage, and (e) avoidance of injuries during cultivation.

11. Wax and certain antiseptics have been shown to exert a marked preservative effect on various wrappers commonly used in grafting work. It is probable that such materials may in this way indirectly play a considerable part in controlling wound overgrowths. Some of the antiseptics employed have also reduced wound overgrowth development by injury to the lower cion tip and callus.

12. A survey made in representative nurseries in the North-Central and Northeastern United States showed that crowngall on apple was widely distributed but was much less prevalent than wound overgrowth. Wound overgrowth on apple was found to be commercially controlled in three nurseries where appropriate grafting methods were used and quite generally where the stock was propagated by budding.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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EXPLANATION OF PLATES

Unless otherwise noted all the apple specimens illustrated in these plates are from the Wealthy variety.

PLATE XXXVIII

Crowngall on apple

A.—Natural infection at the union of a two-year-old grafted tree.

B.—Crowngalls which resulted from inoculations on the cion of a Wealthy apple graft at the time of planting. Photographed after one season of growth. A small gall appears directly below the larger one.

C.—Crowngall which resulted from inoculation on a three-year-old Jonathan apple tree. Photographed six months after inoculation. The wound was made by a cut which extended half way through the stem with an upward inclination. To prevent direct healing a strip of medical adhesive plaster was placed in the cut. Although the stimuli for callus as well as for crowngall production appear to have been present the external characters are those of crowngall. This enlargement should be compared with those shown in Plate XLV, D and Plate XLVI, A.

It is noteworthy that these representative crowngalls are comparatively large in relation to the diameter of the trees, and no roots appear from their surface.

PLATE XXXIX

Wound overgrowths and crowngalls on three-year-old fruit trees

A.—Section through an apple tree showing a wound overgrowth which probably developed after an injury at a. The surface bears root primordia and small roots.

B.—Surface of the wound overgrowth shown in A.

C.—Section at the union of a grafted apple tree showing a wound overgrowth about the lower tip (a) of the cion.

D.—Section through an apple tree showing a wound overgrowth which probably followed an injury evidenced by the dark area at a. Numerous small roots have developed from the surface.

E.—Section through a Bradshaw plum tree showing a crowngall which probably resulted from infection in an injury at b. The soft portion of the gall appears dark because of a brown color due to oxidation which occurs rapidly in such tissue. A considerable area of vascular tissue appears (a), making this specimen of crowngall very hard.

F.—Section through an apple tree showing a crowngall which probably resulted from infection through a wound evidenced by the dark area at b. Vascular elements appear at a, but the outer portion of this gall was comparatively soft. In both E and F no roots appear from the surface of the crowngall proper.

PLATE XL

Surface and sectional views of wound overgrowths at the union of three-year-old grafted apple trees

A.—Surface view of the union. Roots and root primordia are present on the surface.

B.—Section of the enlargement shown in A. The lower cion tip (b) of the original graft was too long. Small dark spots may be seen (a) which commonly yielded bacteria in cultural examinations. These, however, were not the crowngall organism. Compare with Plate XLII, C. The evidence now available indicates that these bacteria have little if any influence in the development of the enlargement.

C.—Surface view of the union. Roots and root primordia are present on the surface.

D.—Section of the enlargement shown in C. The lower tip of the cion (b) extended beyond the cambium (a) on the stock and wound overgrowth developed. Small

cavities and fissures (c) may be seen which often contained soil particles and in culture yielded bacteria which seemed not to be pathogenic.

PLATE XLI

Sectional and surface views at the union of one-year-old apple trees

A.—Section showing the relative positions of the lower cion tip (a) and the exposed cambium of the stock (b) of the original graft. The cion tip has been pushed away from the stock by a cushion of callus tissue.

B.—Section through a smooth union. The lower cion tip has been pushed slightly away from the stock by the cushion of callus (a). Here it may be seen that the upper tip of the stock (b) may be long without any significant influence on the development of wound overgrowth.

C.—Similar to A.

D.—Surface view of a tree similar to that shown in A.

E.—Surface view of a tree similar to that shown in B.

F.—Surface view of a tree similar to that shown in C.

PLATE XLII

Photomicrographs of crowngall and callus tissue of apple. Material of both kinds was collected approximately six weeks after the malformations were initiated

A.—Section through crowngall tissue showing that no definite bark layer is formed at the surface (a) but that the outer part may be covered by dead cells. Two separate layers of cells suggesting cambium may be seen, one deeper in the gall than the other, at b and b′. Enlargement appears to occur by growth of inner tissue.

B.—Section through crowngall tissue showing regions of hyperplasia (a and a′).

C.—Section through a small cavity such as may be found both in crowngall and in callus tissues. Such cavities may contain soil and bacteria which appear to have little if any influence in the development of the enlargement. See Plate XL, B, a, D, c.

D.—Section through part of a callus showing parenchymatous tissue, the cells of which later develop secondary thickening in the walls.

E.—Section through callus tissue showing that the surface layer is comprised of living and actively growing cells (a). Later a phellogen and cambium are differentiated and growth appears to take place by secondary thickening such as occurs in stems.

PLATE XLIII

Surface views of the unions of different types of inferior commercial apple grafts

A.—The cion tip (a) is "short." The exposed cambium of the cion does not extend down as far as that on the root.

B.—The cion tip (a) is "even." The exposed cambium of the cion is opposite that of the root. However, in these cases, due to the type of cut and wrapping, the two cambial layers are not held in close contact. The wrapping thread (b) passes under the lower cion tip and hinders contact of the cambial layers. Grafts in which the lower cion tip stands away from the stock are classed as "loose."

C.—The cion tip (a) is "long." The exposed cambium of the cion extends down beyond that of the root. The pin (b) is opposite the lower extremity of the exposed cambium of the root.

D.—The cion tip (a) is "blunt." These grafts were like those shown in C but the long lower cion tip has been cut off.

E.—The cion is smaller than the stock. The lower cion tip (a) is "short."

F.—The cion is larger than the stock. The lower cion tip (a) is "even." A considerable portion of exposed cambium of the cion is not in contact with that of the root. An exposed portion of the tongue is shown at b.

G.—A representative commercial graft like those in C after being packed in moist sawdust for six weeks. Excess callus has developed at the lower cion tip (a).

H.—A graft which shows the development of a number of roots at c on the stock. This may be the early stage of a development of hairy root like that seen in Plate XLV, A, B, C.

PLATE XLIV

The formation of callus on apple cion wood

A.—Cion wood cut to a feather edge and placed in moist sawdust for two months. Callus has developed along the entire cambial surface but particularly at the lower tip.

B.—Grafts with long lower cion tips photographed two months after planting. Considerable excess callus has developed.

C.—Grafts similar to those in B except that the lower cion tip was properly fitted. No excess callus developed.

The grafts shown in B and C were made by a technique designed to exclude the crowngall organism and planted in steamed soil.

PLATE XLV

Malformations which developed on apple grafts and trees planted in steamed soil

A, B, and C.—Hairy root on one-year-old apple trees. These roots originated on the stock. Compare with Plate XLIII, H.

D.—Wound overgrowths and hairy root which developed from slanting cuts in three-year-old Jonathan trees. Photographed six months after the injury. A piece of medical adhesive plaster was placed in each cut to prevent direct healing.

E.—Wound overgrowths which developed at the union of poorly fitted grafts. Photographed after one growing season.

Cultures of all the enlargements shown on this plate were negative for the crowngall organism.

PLATE XLVI

Callus and wound overgrowths on apple trees grown in steamed soil

A.—Similar to Plate XLV, D, except that the photograph was taken two and a half months after the injury. The callus had not yet passed into the wound overgrowth stage. This callus was produced under conditions designed to exclude the crowngall organism.

B.—Wound overgrowths at the union of a poorly fitted graft. Photographed one year after planting. The enlargement marked a is at the lower cion tip and that marked b is on the cion above the upper end of the stock.

C.—Wound overgrowth at the lower cion tip (a). This is typical of the large wound overgrowth of tables 3, 4, and 5. This enlargement developed from a graft like those shown in Plates XLIII, C, and XLIV, B. Photographed after one growing season.

D.—Wound overgrowth at the lower cion tip (a). This is typical of the small wound overgrowths of tables 3, 4, and 5. Photographed after one growing season.

PLATE XLVII

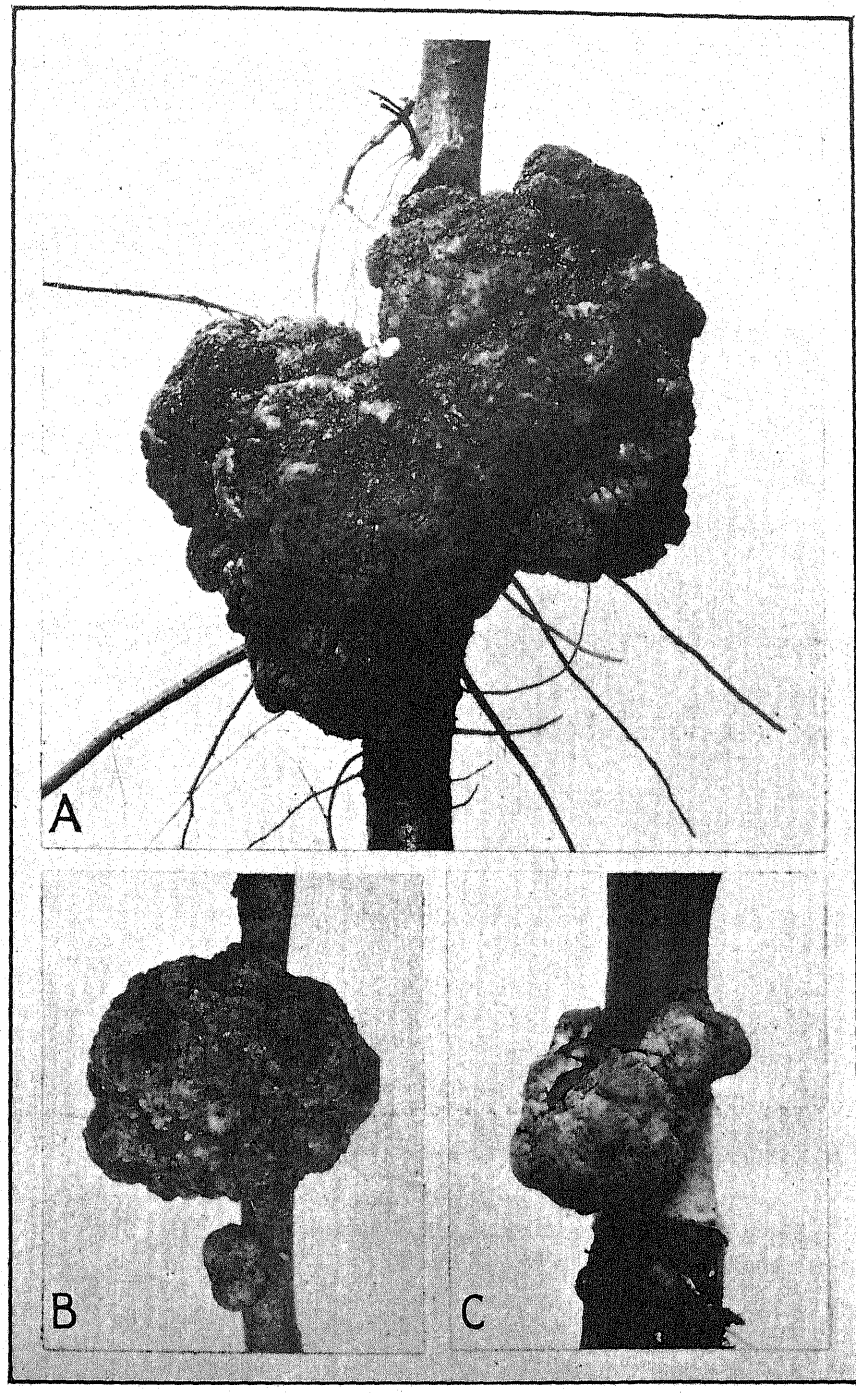
Smooth and malformed unions of apple trees

A.—A smooth union which was wrapped with adhesive plaster which extends from a to b. Roots may be seen piercing the plaster. The cracks in the plaster were produced by expansion of the tree after the plaster rotted. Photographed after one growing season.

B.—Section at the union of a three-year-old tree. The lower cion tip (a) has produced large roots (c and c′) which have assumed much greater importance than the stock (b) in the growth of the tree.

C.—A smooth union on a three-year-old tree. The lower cion tip may be seen at a. In this case the root has developed a greater diameter than the cion.

D.—Hairy root which has developed from the wound overgrowth produced on a graft in which the cion was larger than the root, as shown in Plate XLIII, F. Photographed after one growing season.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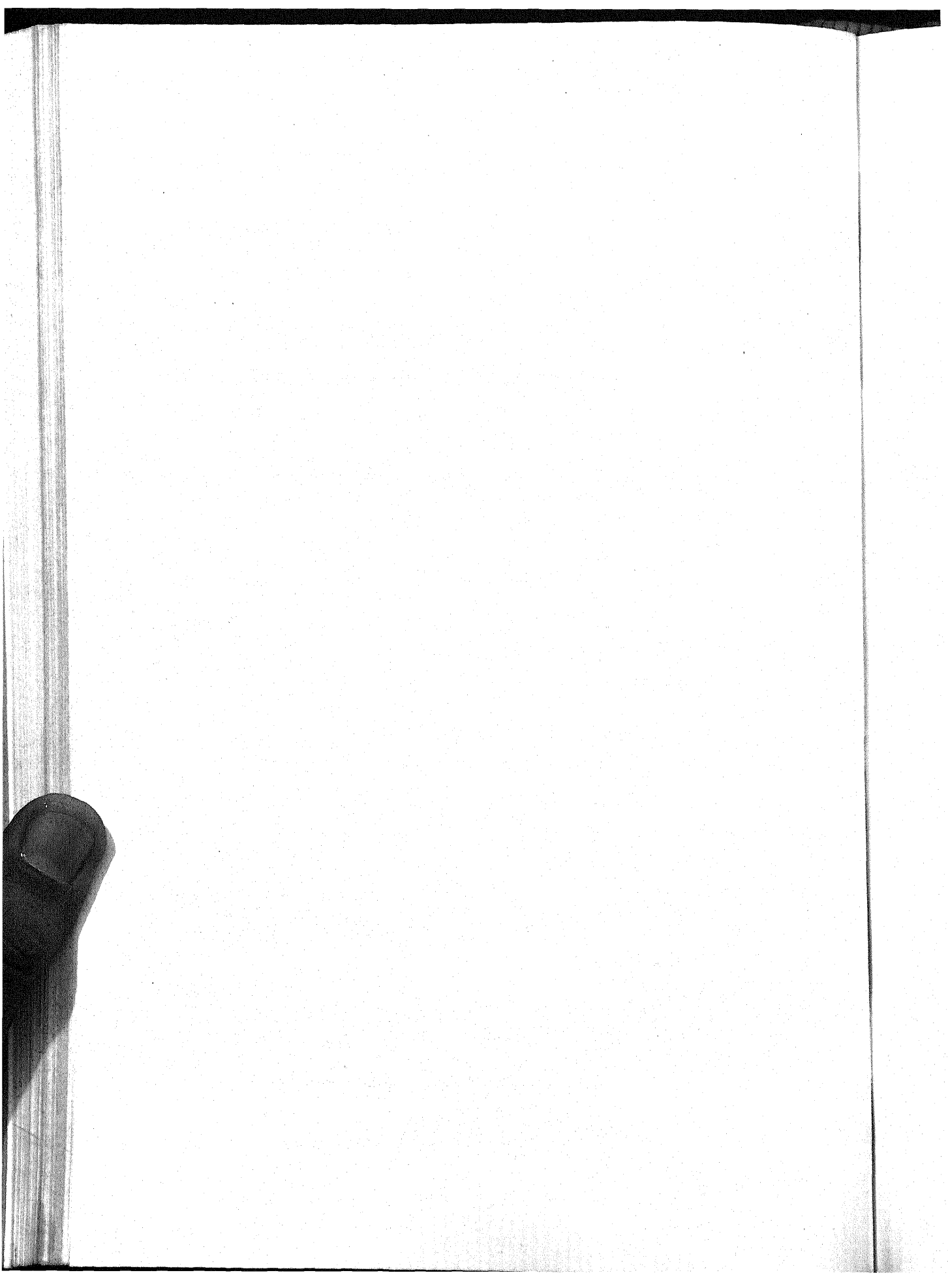

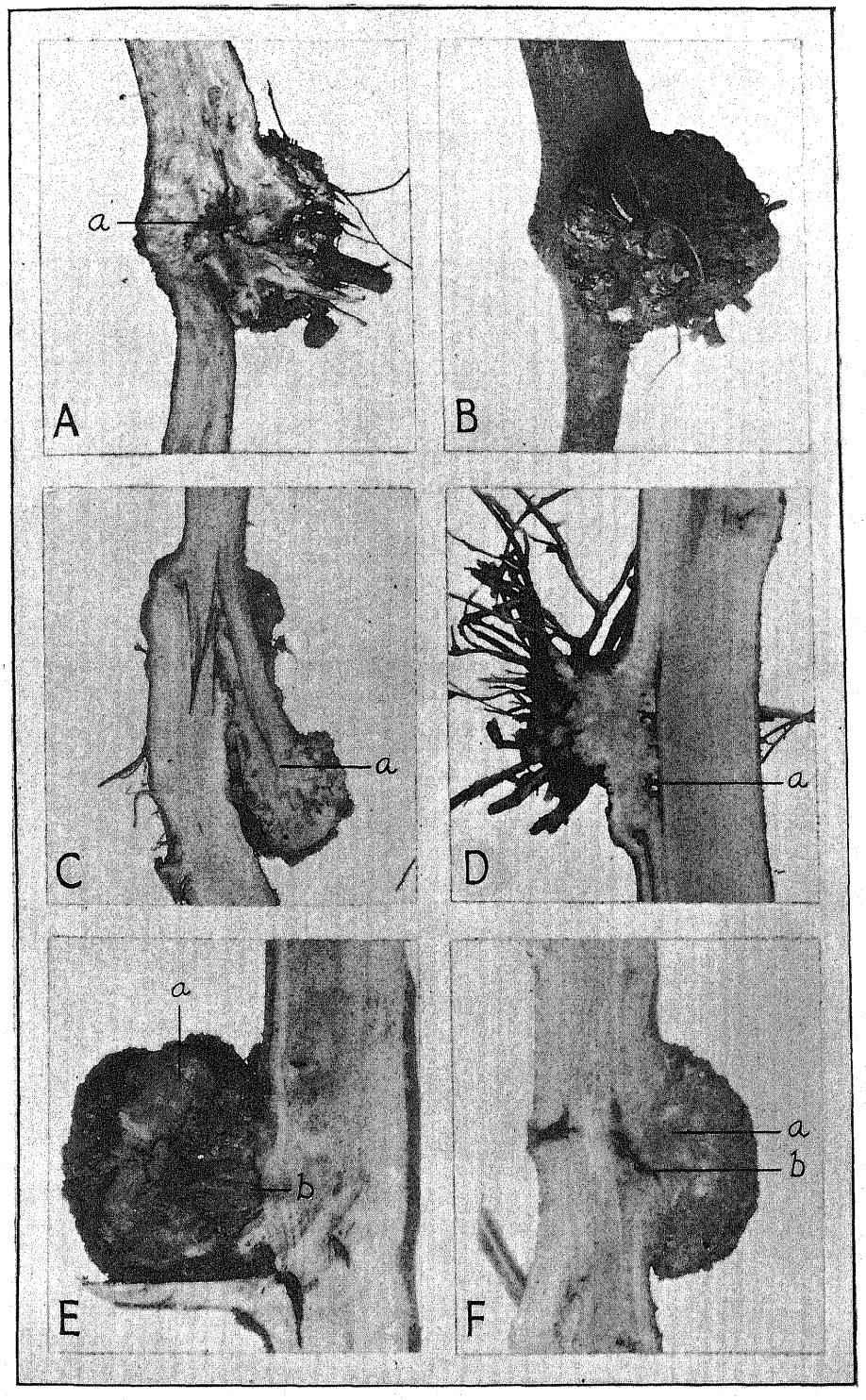
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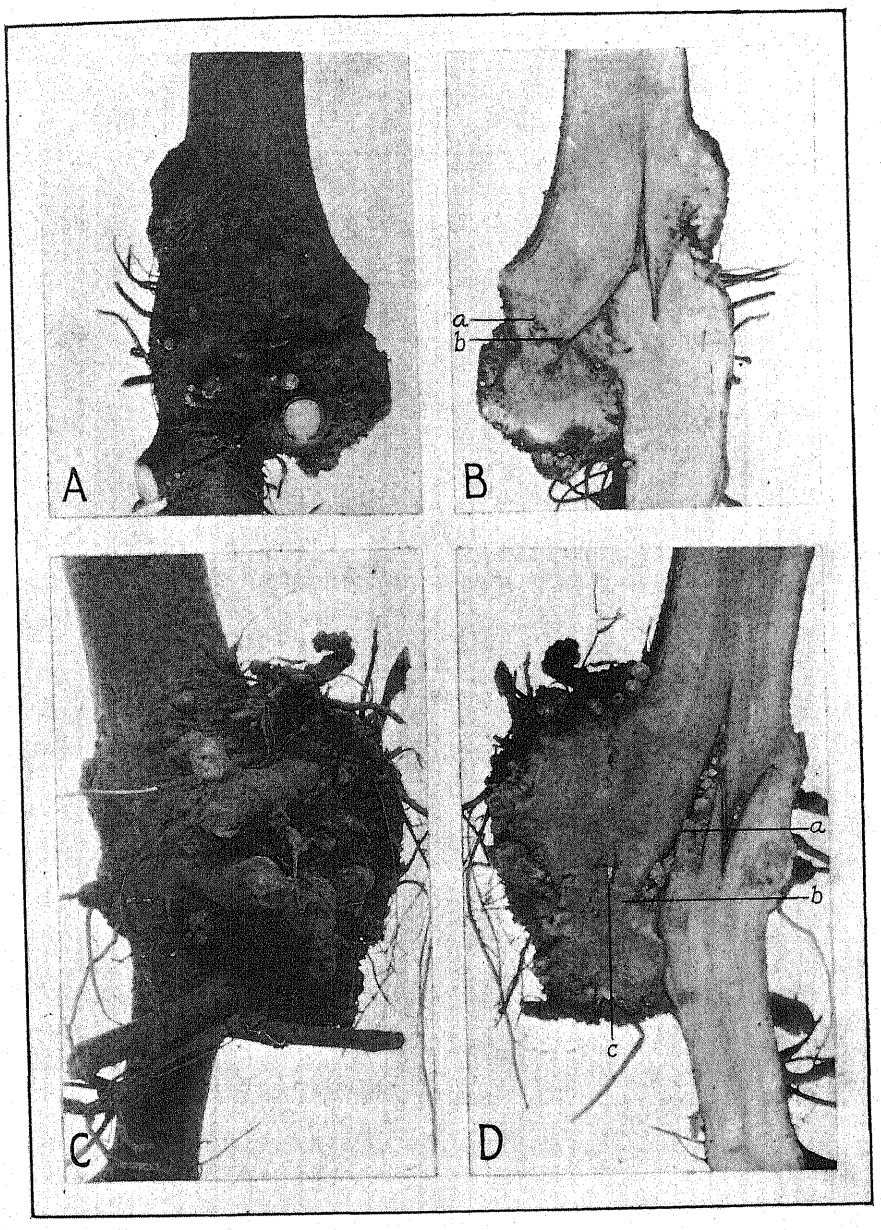
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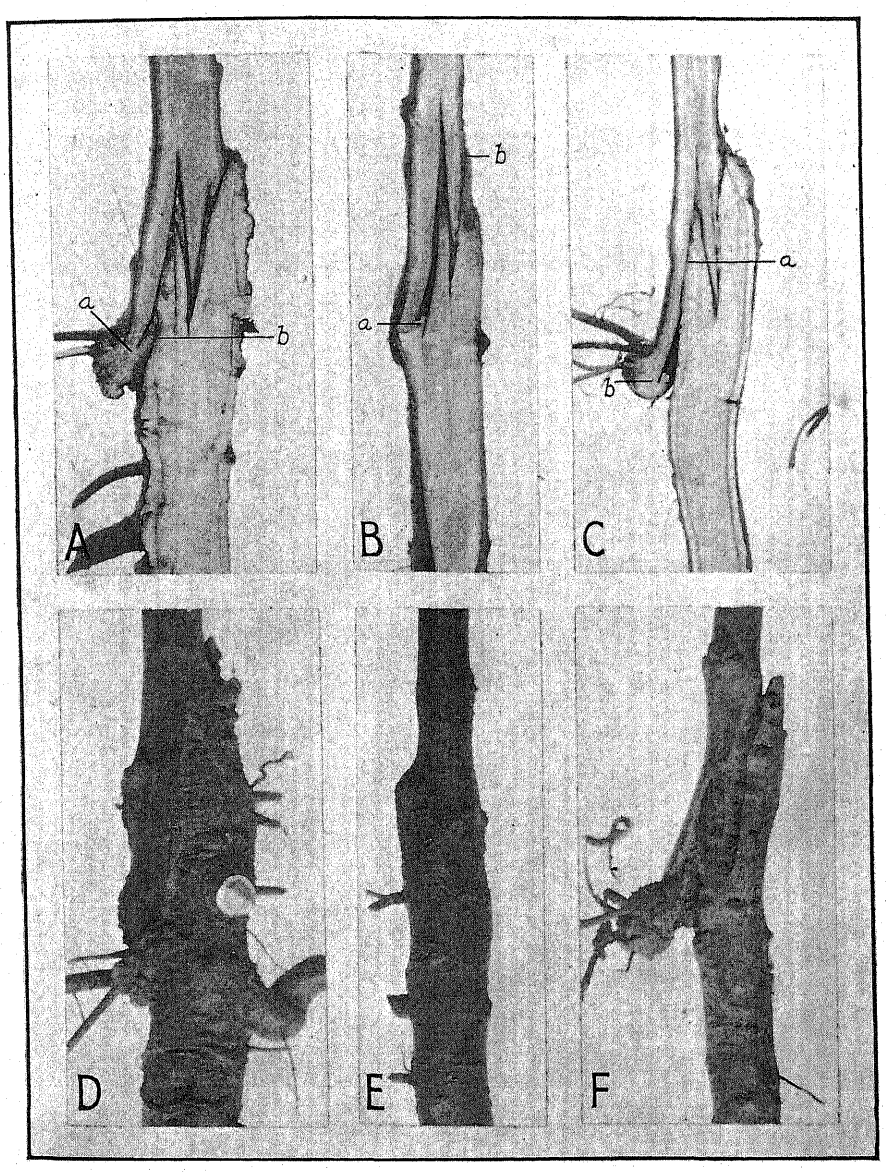
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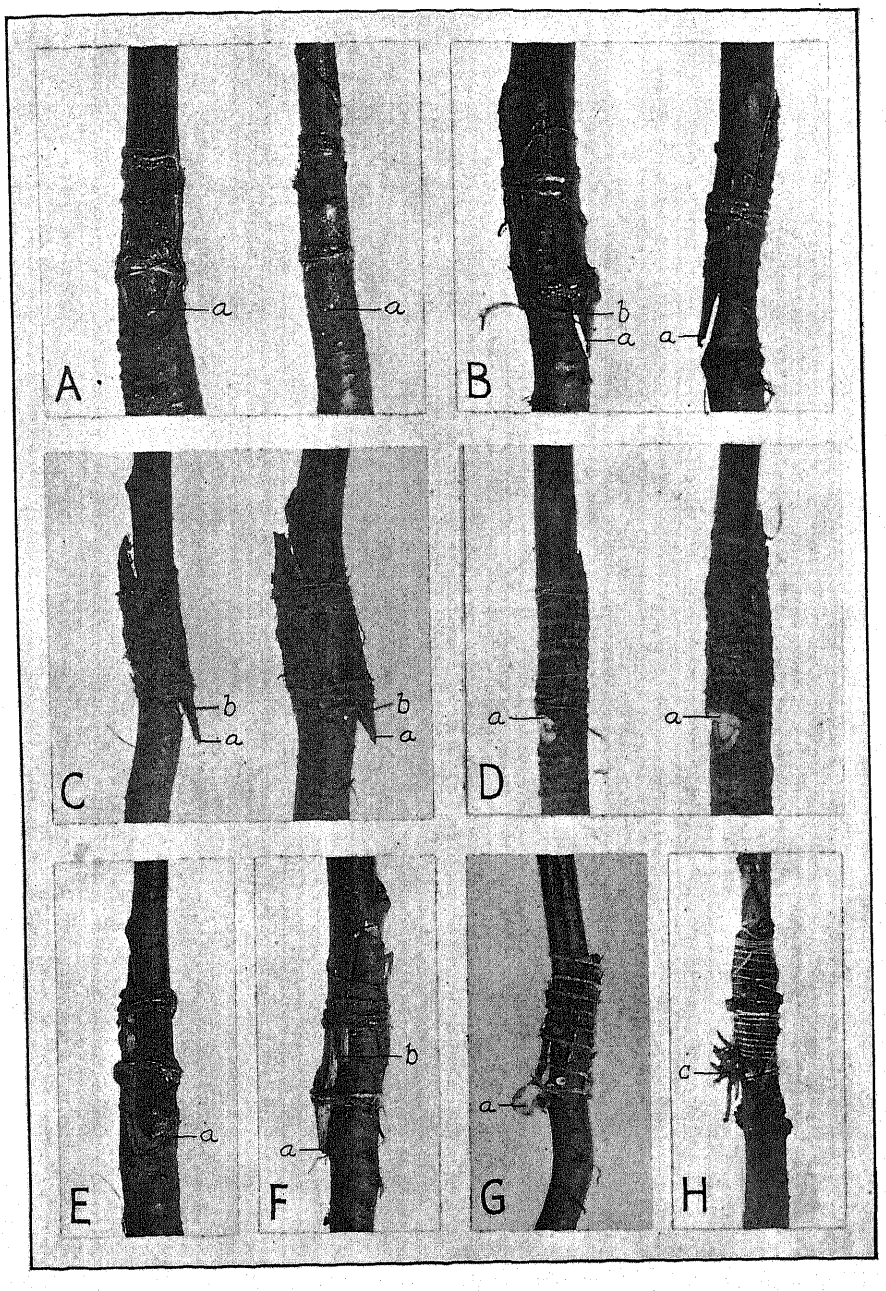
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a
a
B
b
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C
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b
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D
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E
a
F
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G
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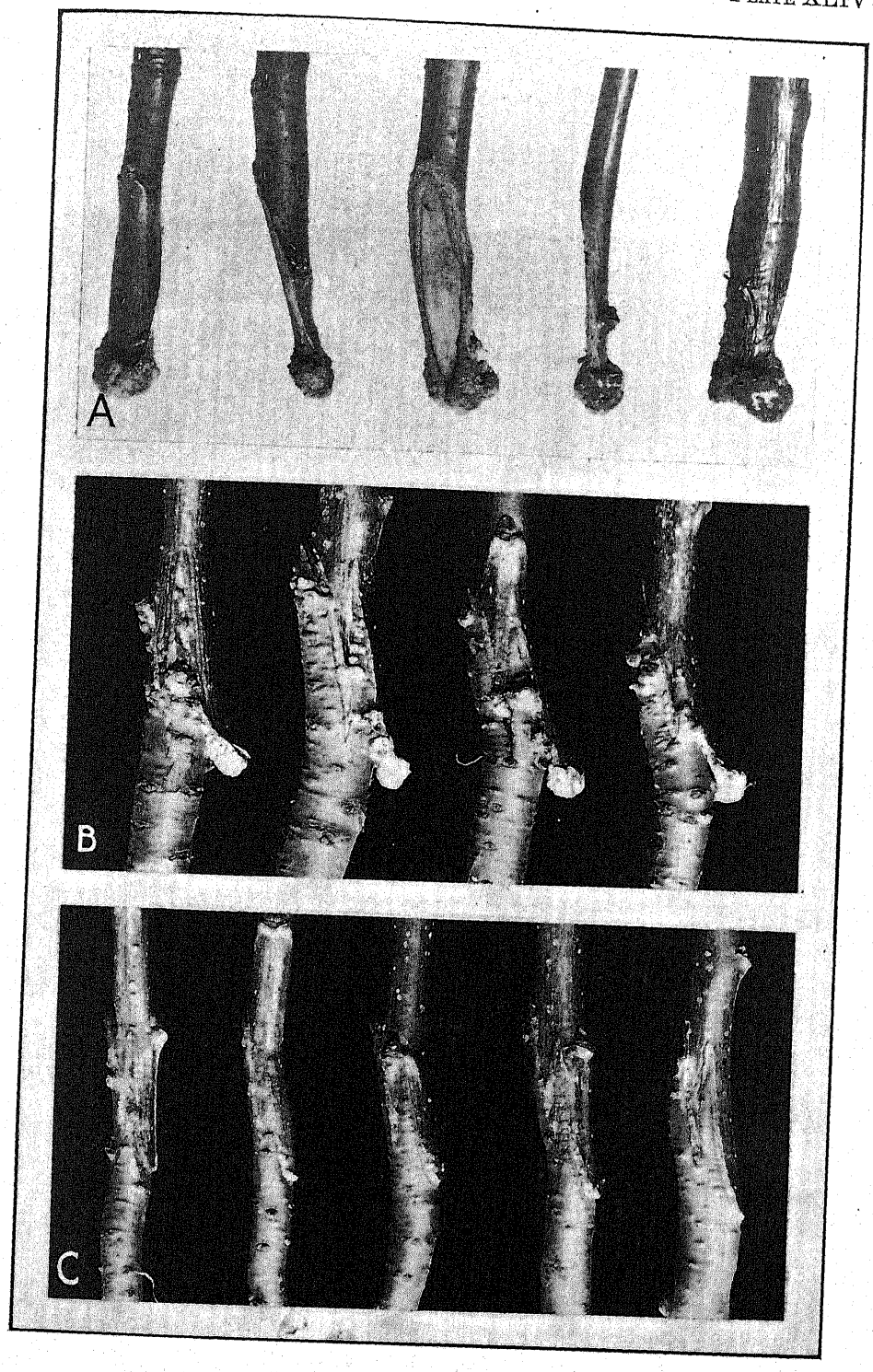
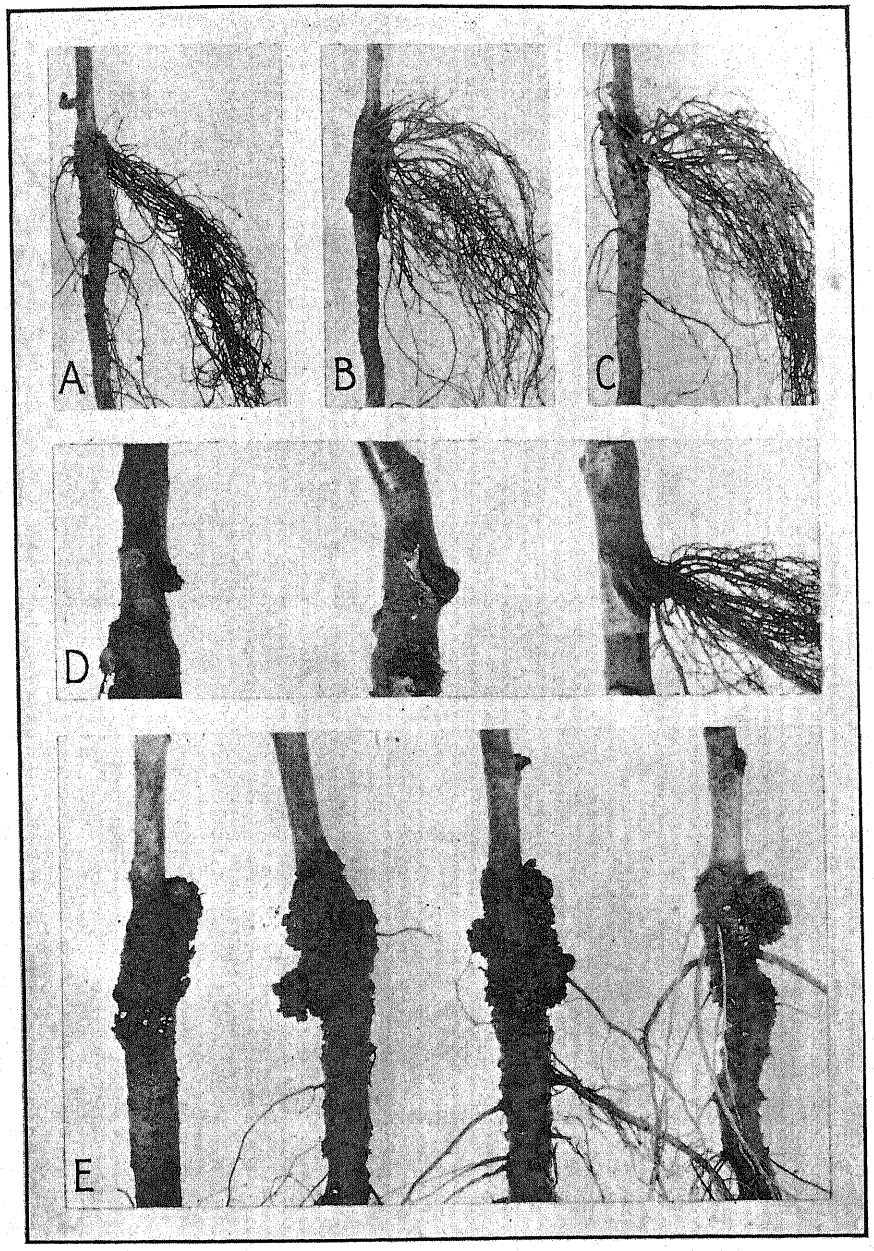
H
c

PLATE XLIV

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B
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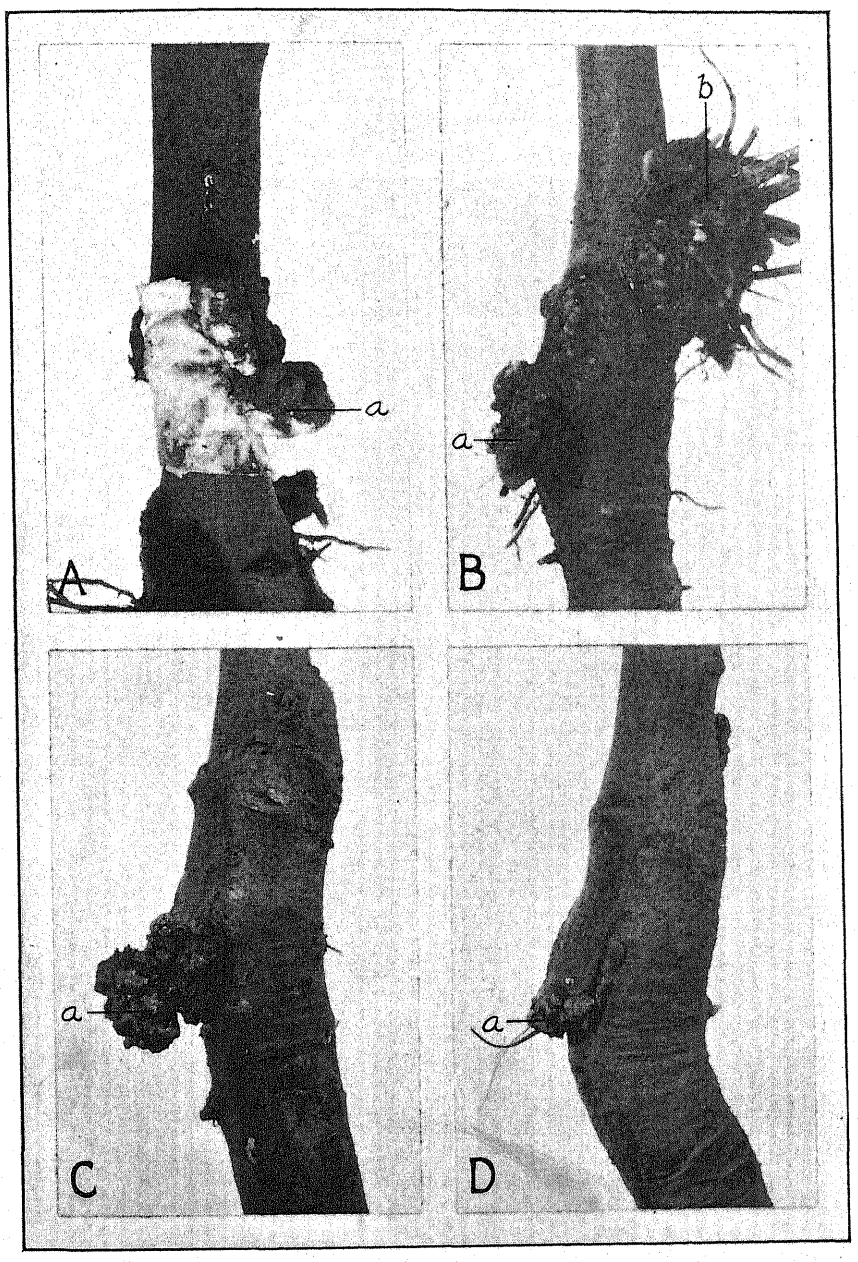
a
A
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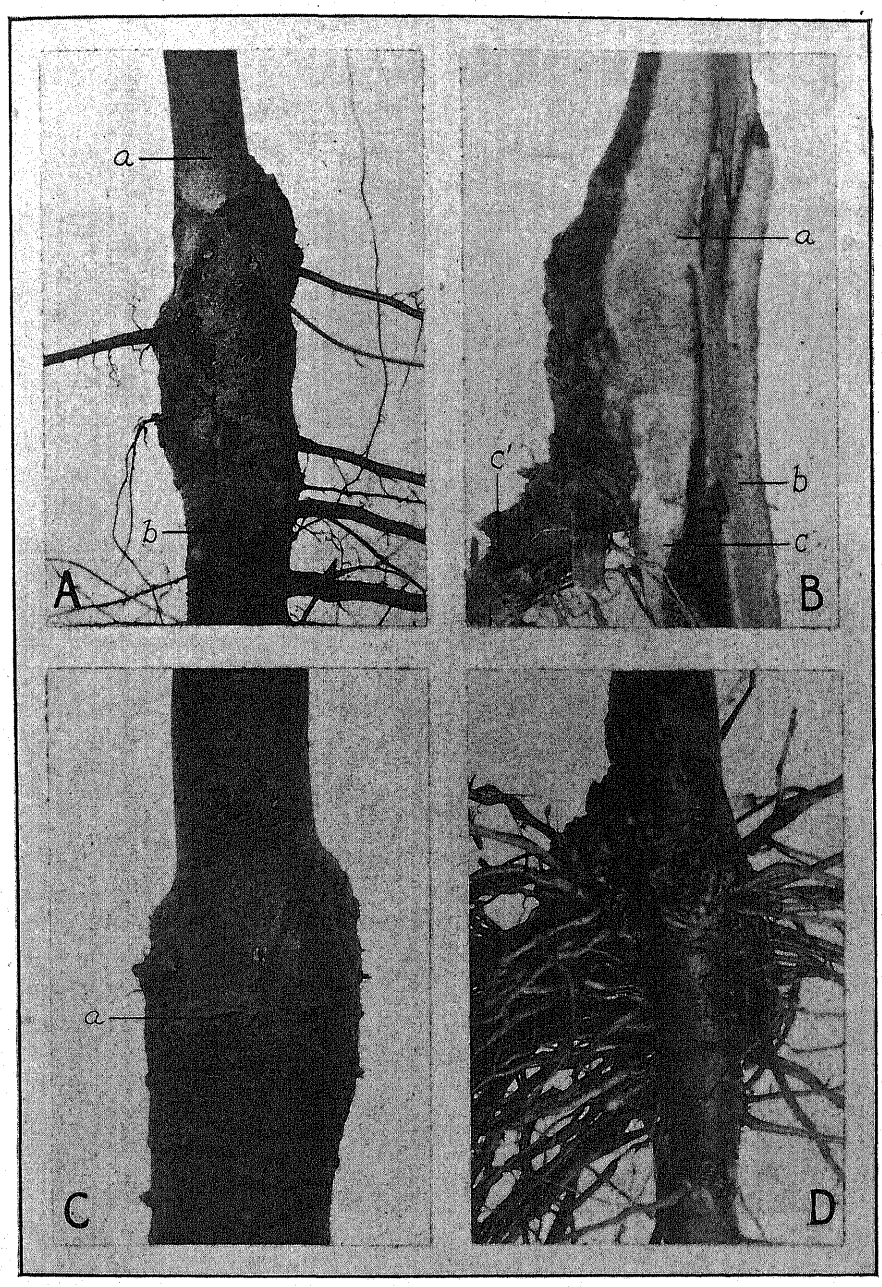
C
a
D


a
b
A
a
c'
b
c
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a
C
D

STUDIES ON THE REACTION TO STEM RUST IN A CROSS BETWEEN FEDERATION WHEAT AND KHAPLI EMMER, WITH NOTES ON THE FERTILITY OF THE HYBRID TYPES

H. J. Hynes[1]

It is generally recognized that the production of varieties of wheat resistant to stem rust (*Puccinia graminis tritici* Erikss. and Henn.) is one of the most hopeful methods of minimizing losses from rust epidemics. This is a complex problem, but investigations on the parasitism of the rust fungus, together with the inheritance studies on resistance to rust in certain crosses, have elucidated the problem considerably.

The extensive researches of Stakman and his co-workers (8, 12, 14, 15) have shown that *Puccinia graminis tritici* consists of 37 or more physiologic forms which can be differentiated by their reaction on 12 varieties of wheat. It is clear, therefore, that if a variety of wheat is classed resistant to stem rust it must be resistant to all the physiologic forms which occur in the particular locality where it is grown. It is evident also that a wheat may be resistant in one region but quite susceptible in another. The problem is still one of developing commercial varieties which are resistant to all forms of rust.

KHAPLI IN RELATION TO STEM RUST

There is only one wheat known, Khapli, C. I.[2] 4013, (15) which is resistant to all known physiologic forms of stem rust. Many attempts (4) have been made to secure a fertile cross between Khapli and Marquis, C. I. 3641, in connection with rust breeding work. These crosses did not appear at all promising, owing to the high degree of sterility and extreme variability of the hybrids.

The present paper deals with studies on a cross between Federation, an Australian mid-season variety of *Triticum vulgare,* and Khapli, an early variety of *T. dicoccum.* The main purpose is to present the results of a study of the reaction of the F_3 generation of the cross to two physiologic

[1] Walter and Eliza Hall Agriculture Research Fellow, University of Sydney, Australia, and Graduate Student of the University of Minnesota.

[2] C. I. = Accession number of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

forms of *P. graminis tritici*. A classification of the F_2 and F_3 plants, together with the percentage fertility of each, is given in addition to a brief statement on the reaction of certain F_4 families to several forms of rust in the rust nursery, University Farm, St. Paul.

WHEAT SPECIES CROSSES IN RELATION TO STEM RUST

The work of various investigators, Tschermak (16, 17), Kezer and Boyack (6), Freeman (1), Hayes *et al* (2, 5), carried on through the period from 1913 to 1924, contributed results which give a clearer understanding of the species groups in wheat and the difficulties of making successful crosses between them.

Plant breeders are particularly interested at the present time in the possibility of transferring rust resistance from the emmer group to the vulgare group. Freeman and Johnson (4), in 1908, made crosses between varieties of *T. durum* and *T. vulgare* and found it difficult to combine the rust-resistant qualities of the durum parent with the agronomic characters of the common types.

Hayes, Parker, and Kurtzweil (2) studied the inheritance of reaction to stem rust in crosses between *T. vulgare* and *T. dicoccum* and between *T. vulgare* and *T. durum*. They found partial linkage between rust resistance and the emmer and durum types and that it was difficult to secure types which were common and resistant. The fact that some crossing over occurred shows that by growing large numbers of plants in the F_2 and F_3 generations it is possible to obtain rust-resistant common wheat types.

The problem of developing rust-resistant common types from crosses between wheat species of different chromosome number is very complex. In such crosses there is considerable sterility in the resulting progeny. The amount of sterility depends upon the compatibility of the chromosomes of the parents. Cytological studies by Sax (10), Kihara (7), and Watkins (19) have shown that sterility in wheat species hybrids may be due in part to gametic sterility resulting from the proportion of univalent to bivalent chromosomes and in part to zygotic sterility which may result in complete failure of the zygote to function. The possible elimination of certain gametic or zygotic combinations lessens the possibility of obtaining desirable types from species crosses.

The more recent work on wheat species crosses has demonstrated a correlation between chromosome numbers and essential species characters. From his studies on crosses between varieties containing 42 chromosomes (*T. vulgare*) and varieties containing 28 chromosomes (*T. durum*), Sax (11) concludes that it is practically impossible to transfer rust resistance from the emmer group to the vulgare group, since durum characters are

strongly correlated with the 28 chromosome number. In other words, the seven additional univalents contributed by the vulgare parent are responsible for the parent characters, and, since susceptibility to rust is a vulgare character, all segregates which have 42 chromosomes must also be susceptible to rust.

There is evidence to show that this conclusion is not of general application. In the Marquis × Iumillo cross Hayes *et al* (5) have shown that the rust resistance of the durum parent was transferred to a type which had 42 chromosomes and also the characters of the vulgare group. Similar results were obtained in Marquis × Mindum and Marquis × Velvet Chaff crosses. From a cross of Marquis × Iumillo with Marquis × Kanred it appeared that two chromosomes or parts of chromosomes which contained factors for resistance were obtained from the durum parent and were combined with chromosomes of the vulgare group.

These results indicate the possibility of transferring the resistance to stem rust from the 28-chromosome group to the 42-chromosome group. This of course must be difficult since resistance is not only determined by several factors, but chromosome irregularities enter in, which make the obtaining of homozygous individuals of the desired genetic constitution much more difficult.

MATERIALS AND METHODS

In the spring of 1921 the writer crossed Federation wheat (*Triticum vulgare*) and Khapli emmer (*T. dicoccum*). For this work, plants of the parent varieties were made available by Mr. W. L. Waterhouse in the experimental plot, Sydney University, New South Wales, Australia. The grain from which the sowings of these varieties were made had been secured from Mr. J. T. Pridham, Plant Breeder at the Experiment Farm, Cowra, and was labelled "pure line" seed.

The usual technique was employed in making the crosses (3). As a result of artificially pollinating about 15 emasculated flowers of Federation wheat with pollen taken from the Khapli parent, five somewhat shrivelled grains were produced. The F_1 plants, four in all, were quite normal and produced heads bearing plump grain. The heads were intermediate for those characters which separate the parental types. The F_2 grain was also sown in the experimental plot at Sydney University, and in December, 1923, 42 F_2 plants were harvested. Some plants died in the early stages, and others lived until the early boot stage and then died. Studies on the F_2, F_3, and F_4 generations were made at the Section of Plant Pathology, University of Minnesota.

The F_2 generation consisted of plants of very different types which were classified with the assistance of Dr. H. K. Hayes of the Section of Plant

Breeding (Plates XLVIII and XLIX). The percentage fertility was then determined on the basis of the number of seeds set per spike. A portion of the grain from each plant was reserved for planting in the field at University Farm, St. Paul, while the remainder was sown in the greenhouse in connection with a study on the reaction of the F_3 generation to two physiologic forms of stem rust.

The soil used for the greenhouse experiments was steam sterilized before potting in order to avoid injury to the seedlings by root-rotting fungi. Five seeds were sown to a pot. The seedlings were grown during the summer of 1924 in a greenhouse in which there were no rust-infected plants. When they were about 3 inches high, they were inoculated with urediniospores of form 15 and then incubated in a moist chamber for 48 hours. At the end of this time they were placed on well-lighted greenhouse benches where the temperature and moisture conditions were kept as uniform as possible. The methods used in inoculation were the same as those employed by Stakman and Piemeisel (13). Control plants of the parent varieties were inoculated at the same time as each set of hybrids.

Notes on the types of infection with both physiologic forms were taken from 18 to 20 days after the date of inoculation. It was found that environmental conditions influenced to some extent the amount and rapidity of rust development, but the character of infection was always fairly constant regardless of external factors.

After notes on the infection produced by the first form had been recorded, the infected leaves were removed and the plants thoroughly sprayed to remove any remaining spores. These plants were then set aside, and, when the next leaf emerged, were inoculatd with form 27. After incubation they were placed in an adjoining section of the greenhouse where there were no rust-infected plants. Thus there was practically no chance of accidental contamination.

A higher percentage of infection was obtained with form 15 than with form 27. This apparently was due to essential differences between the first and second leaves rather than, as Puttick (9) suggests, to the difficulty of keeping a film of moisture on the second leaf during the incubation period. With special attention a completely saturated atmosphere was maintained during this period. On removal from the incubator there was a film of moisture over the entire leaf surface.

In recording the types of infection the symbols prepared by Stakman and Levine (15) in their work on physiologic specialization were used (Table 1).

TABLE 1.—*Explanation of symbols used to indicate types and degrees of infection produced by physiologic forms of Puccinia graminis on varieties of wheat*

Types of infection

0—IMMUNE

No uredinia developed; hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasion in the host tissues.

1—VERY RESISTANT

Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive, necrotic areas.

2—MODERATELY RESISTANT

Uredinia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green, but slightly chlorotic, islands.

3—MODERATELY SUSCEPTIBLE

Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.

4—VERY SUSCEPTIBLE

Uredinia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present under certain conditions.

X—HETEROGENEOUS

Uredinia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill defined.

Degrees of infection

(=)—TRACE

Uredinia very few in number; development of rust generally poor and decidedly subnormal.

(—)—SLIGHT

Rust development below normal, but somewhat better than "trace."

(±)—MODERATE

Variation in rust development from "slight" to "considerable"; when infection is uniform but only medium in quantity the symbol is omitted.

(+)—CONSIDERABLE

Infection better than normal; uredinia fairly numerous and scattered.

(;)—FLECKS

Hypersensitiveness with no uredinia.

In the rust reactions recorded for the hybrid material the symbols n–, n, and n+ were included under each degree of infection in the moderately susceptible and very susceptible classes. This referred to the degree of necrosis; n– indicated very little necrosis; n, moderate necrosis; and n+, very pronounced necrosis.

PHYSIOLOGIC FORMS USED

In June, 1924, there were about 14 physiologic forms of *P. graminis tritici* available in the greenhouse. Although the reaction of Khapli, C. I. 4013, to these forms was known, it was necessary to know the reaction of Federation, C. I. 4734, before selecting forms for test on the hybrids. Table 2 gives the average types of infection obtained with several forms on Federation and Khapli.

TABLE 2.—*Reaction of Federation and Khapli to ten physiologic forms of Puccinia graminis tritici, expressed by type of infection produced*

Variety	Type of infection produced by physiologic form									
	1A[a]	3A[a]	9C[a]	15	17A[a]	19	21	27	33	38
Federation ...	4–	4	4–	4+n–	4	4	4+	4–n+	4+	4+
Khapli	1–	1	1	1=	1–	1–	1–	1+	1	1–

[a] Single spore cultures kindly made available by Dr. M. N. Levine. Arabic figures indicate form numbers; capital letters designate different monosporous cultures.

It was not possible to select forms which reacted reciprocally on the parents of the hybrids, since Khapli is resistant to all known forms. Forms 15 and 27 were selected. The former is strongly parasitic on all the "differential hosts" except Khapli; the latter is a less virulent form.

Form 15 was collected on wheat at Pusa, India, in March, 1923. Form 27 was likewise collected in India in February, 1923, and was definitely identified in October of the same year. When used in the present investigation, both forms had been cultured through approximately 20 uredinio-spore generations without any indication of a change in their parasitic behavior.

BASIS FOR INTERPRETING RUST REACTIONS

The results obtained were interpreted largely on the basis of the reactions of the parental varieties to forms 15 and 27. It was considered that all plants which gave a reaction of from 0 to 3= were resistant; plants which showed a high degree of hypersensitiveness in the 3+ classes were placed in the moderately resistant class. In fact it seems reasonable to assume that plants falling in the 3+ class with sharp necrosis were just as resistant as those falling in the 3 or 3– classes without necrosis. Admit-

tedly the line of demarcation between susceptibility and resistance is an arbitrary one, but for the purpose of separating resistant and susceptible families from the plant breeding standpoint the scheme adopted seems to be warranted.

EXPERIMENTAL RESULTS

THE F_2 PLANTS OF FEDERATION × KHAPLI

The data taken on the F_2 plants are summarized in table 3. The hybrids were classified on the bases of prominence of keel, compactness of head and brittleness of the rachis. As already indicated, the percentage fertility was determined on the basis of the number of seeds set per spike.

The F_2 plants were either of the emmer, durum, vulgare or intermediate types. The percentage fertility varied considerably, ranging from 0 to 100 per cent. These results are in accord with those obtained by other workers on emmer-vulgare crosses. The F_1 is usually partially sterile, while in the F_2 variation occurs between complete fertility and complete sterility; the plant characters are those of the parent varieties, or intermediate between them.

TABLE 3.—*Classification and percentage fertility of individual F_2 plants of Federation × Khapli*

Type	Percentage fertility	Type	Percentage fertility	Type	Percentage fertility
Vulgare	37	Intermediate	41	Emmer	6
do	22	do	40	do	6
do	1	do	39	do	5
Intermediate	100	do	38	do	5
do	93	do	26	do	2
do	76	do	17	do	2
do	73	do	8	do	1
do	72	do	4	Durum	59
do	65	do	0	do	58
do	63	Emmer	95	do	46
do	63	do	46	do	40
do	58	do	26	do	39
do	46	do	16	do	24
do	41	do	11	do	3

RESULTS OBTAINED FROM INOCULATING F_3 SEEDLINGS WITH FORMS 15 AND 27

The types of infection obtained on the F_3 seedlings with forms 15 and 27 are summarized in tables 4 and 6 respectively. In general, the percentage of infection obtained was quite satisfactory, although in a few families

there is evidence of little infection. Many plants which gave a "O" reaction from the first inoculation were again inoculated to ascertain whether they were really immune. Many of these proved to be susceptible. A fairly large percentage of plants inoculated with form 27 escaped infection. This apparently was due to the fact that the leaves inoculated with this form had reached a stage of development which was unfavorable for optimum infection. In analyzing the results those plants which appeared immune (O reaction) were disregarded.

A study of the infection data showed a very wide range in type of infection. Some families exhibited practically all types and degrees of infection, together with varying degrees of necrosis. In recording the results it seemed that possibly there were genetic factors which determined pustule development and others, again, which determined the degree of hypersensitiveness. Some plants in certain families developed the parental types of infection, and between these extremes were plants which showed all gradations of infection. These were apparently intermediates. Plants inoculated with form 15 obviously could not give reactions which transgressed those of the parental varieties on account of the extremes of susceptibility and resistance of Federation and Khapli respectively. With form 27, however, some plants appeared more susceptible than Federation. In family 41 there were 6 plants out of 58 infected which were more susceptible than the susceptible parent. This may be a case of transgressive segregation.

Out of a total of 1492 F_3 seedlings inoculated with form 15, 1253 were infected; while with form 27, 527 individuals were infected out of a total of 917 inoculated. The results are summarized in tables 4 and 6. This was done according to the following scheme:

RESISTANT.	Plants showing	0;	to 3 =	reactions inclusive.
INTERMEDIATE.	Plants showing	3 −	to 3 + $^{n+}$	do do
SUSCEPTIBLE.	Plants showing	3 + ^{n}	to 4 +	do do

Each family was then classified according to the distribution of infection as follows:

S—Homozygous for susceptibility.
HS—Heterozygous, with susceptibility dominant.
H—Heterozygous. Plants evenly distributed in all classes.
HR—Heterozygous, with resistance dominant.
R—Homozygous for resistance.

From table 4 it is seen that nearly half the number of families fall into the H class in their reaction to form 15. Of the remaining families, 10 are classified as HS, and 3 as HR.

In analyzing the data, it appeared that there were two, and probably three, disturbing factors which precluded an exact genetic interpretation of the results. The first was the low percentage of fertility of certain families. In crosses between varieties of *T. vulgare* and *T. dicoccum* considerable sterility results. This might cause the elimination of certain gametic or zygotic combinations, thus probably eliminating the type desired. Another

TABLE 4.—*Summary of results showing the reaction of the parent varieties and F_3 families of Federation × Khapli to P. graminis tritici f. 15 Stak. and Lvne. and the number of individuals in each class*

Parents or F_3 hybrid families	Reaction of family	Number inoculated	Per cent infected	Resistant	Intermediate	Susceptible	Total
Federation	S	60	95	0	0	57	57
Khapli	R	26	88	23	0	0	23
1	HS	328	86	21	49	212	282
2	HS	83	89	1	8	65	74
3	HS	76	87	7	11	48	66
4	HS	47	85	2	6	32	40
5	H	39	79	8	5	18	31
6	H	4	100	0	4	0	4
7	H	33	97	4	16	12	32
8	H	9	100	5	1	3	9
9	HR	16	100	13	2	1	16
10	HS	208	73	11	19	121	151
11	H	21	81	6	1	10	17
12	HS	25	96	1	2	21	24
13	HS	84	94	7	17	55	79
16	H	5	100	2	0	3	5
18	H	6	100	3	1	2	6
21	H	18	100	3	4	11	18
22	H	7	86	0	3	3	6
23	HS	23	91	1	4	16	21
25	H	3	100	1	0	2	3
26	HR	12	58	3	3	1	7
27	H	28	100	12	7	9	28
28	HS	14	64	2	0	7	9
29	H	15	73	4	3	4	11
30	H	108	88	25	34	36	95
32	H	62	98	18	18	25	61
33	HS	59	90	4	18	31	53
34	H	24	29	2	2	3	7
35	HR	16	81	8	3	2	13
39	HR	27	56	10	2	3	15
41	H	92	76	12	27	31	70

factor is the possibility of cross pollination in hybrids. Hayes, Parker and Kurtzweil (2) give evidence of natural crossing which occurred in the F_2 generation of a vulgare × durum cross. This fact emphasizes the necessity for caution in analyzing data. Unless special care is exercised, it is probable that the inheritance of certain characters of the hybrid parents might be inaccurately determined. The small number of families and the comparatively small amount of grain available for the greenhouse tests also added to the difficulty in analyzing the rust data.

A larger number of seeds was sown than seedlings inoculated. This was due partly to the failure of some seeds to germinate and partly to Helminthosporium infection which caused some seedlings to die in the early

TABLE 5.—*Reaction of certain F_3 families of Federation × Khapli to P. graminis tritici f. 15 Stak. and Lvne.*

Family	No. of plants as resistant as Khapli	No. of plants more susceptible than Khapli	Calculated and observed ratios	Deviation / Probable error
1	12	270	15:1 63:1	2.1 5.4
2	1	73	63:1	0.22
3	2	64	15:1 63:1	1.6 1.42
13	2	77	15:1 63:1	2.03 1.03
27	7	21	3:1	0.0
32	11	50	3:1 15:1	1.8 5.6
33	3	50	15:1	0.26
41	3	67	15:1 63:1	1.004 2.7

stages of growth. All soil was sterilized before potting; the infection from *Helminthosporium* presumably resulted from grains which showed "black point."

The interpretation of the results in terms of Mendelian ratios is therefore scarcely possible. In any case it is evident that the number of seedlings available in certain families for the rust tests was much too small for a genetic analysis. For this reason, families with less than eight seedlings

were disregarded in the analysis. It is realized that eight plants is too small a number to indicate definitely the genetic nature of the F_2 parent, yet the results obtained are sufficiently reliable to indicate the number of factors involved. On this basis, then, it is evident from table 4 that the ratio is as follows:

0 S : 10 HS : 10 H : 3 HR : 0 R

The plants in family 9 were for the most part resistant; it is possible that the plant which showed a "4" type of infection may have resulted

TABLE 6.—*Summary of results showing the reaction of the parent varieties and F_3 families of Federation × Khapli to P. graminis tritici f. 27 Stak. and Lvne., and the number of individuals in each class*

Parents or F_3 hybrid families	Reaction of family	Number inoculated	Per cent infected	Resistant	Intermediate	Susceptible	Total
Federation	S	32	100	0	0	32	32
Khapli	R	26	96	25	0	0	25
1	HR	110	21	16	6	1	23
2	H	22	73	1	7	8	16
3	HS	21	67	0	6	8	14
4	H	29	72	5	7	9	21
5	H	31	65	5	10	5	20
7	H	19	63	3	5	4	12
8	H	10	60	2	2	2	6
9	H	9	56	1	4	0	5
10	HR	132	35	28	10	8	46
11	H	15	53	3	3	2	8
12	HS	15	87	1	3	9	13
13	H	57	74	6	19	17	42
16	HR	6	50	2	1	0	3
18	H	5	60	2	0	1	3
21	HS	11	91	1	2	7	10
22	H	7	71	2	2	1	5
23	S	13	77	0	0	10	10
26	H	13	69	4	0	5	9
27	H	23	87	3	8	9	20
28	H	12	92	5	3	3	11
29	H	20	90	11	2	5	18
30	H	97	67	32	20	13	65
32	HR	40	70	17	9	2	28
33	H	54	41	6	6	10	22
34	HR	21	67	10	2	2	14
35	HR	10	60	5	1	0	6
37	H	2	100	1	1	0	2
39	HR	22	59	10	1	2	13
41	HS	85	68	6	4	48	58
42	H	6	67	2	1	1	4

from open pollination. Assuming that this is so and that the family is resistant, the ratio would be 22 susceptible to 1 resistant. This could be explained fairly satisfactorily on a two factor hypothesis. There is a deviation from the expected of 0.56 times the probable error which indicates a good agreement between the observed, 22:1, and the calculated 15:1. The fact, however, that none of the families are homozygous for susceptibility or resistance and that there are all grades and intergrades of infection in certain families precludes the probability of such a type of inheritance. The results are explained on the basis of multiple factors.

A study of certain F_3 families in regard to their reaction to form 15 is given in table 5. Considering that susceptibility or an intermediate type of infection is dominant over the Khapli type of resistance, and arranging the material in two classes: 1, as resistant as Khapli, and 2, more susceptible than Khapli, it is possible to approximate roughly the number of genetic factors involved. While this method is somewhat questionable, it gives some idea of the complexity of the results.

TABLE 7.—*Reaction of certain F_3 families of Federation × Khapli to P. graminis tritici f. 27 Stak. and Lvne.*

Family	No. of plants as resistant as Khapli	No. of plants more susceptible than Khapli	Calculated and observed ratios	Deviation / Probable error
4	1	20	15:1	0.41
5	2	18	3:1	2.3
			15:1	1.02
13	2	40	15:1	0.58
27	1	19	15:1	0.34
33	3	19	3:1	1.8
			15:1	2.1
41	2	56	15:1	1.3
			63:1	1.7

No definite conclusions can be drawn from the data presented in table 5. It would appear, however, that in families 2, 27, and 33 the observed and the calculated agree rather well. In families 1 and 32 the observed ratios seem to agree fairly well with the calculated ratios, 15:1 and 3:1 respectively.

An analysis similar to that given for results with form 15 was made for data obtained with form 27. (See also Plates L and LI.) This is tabulated in table 6. Disregarding families with less than eight plants, the ratio is as follows:

1 S : 4 HS : 12 H : 5 HR : 0 R

In family 1 only one plant out of 23 was susceptible. If this family is considered resistant, the ratio 21 susceptible to 1 resistant approximates a 15:1 ratio. There is a deviation from the expected of 0.48 times the probable error. For reasons previously given, however, the reaction of the F_3 generation to form 27 is interpreted on a multiple factor basis.

Following the scheme adopted in table 5, an analysis was made of the reaction of certain F_3 families to form 27. These results are presented in table 7. It would appear that the observed ratios in each of the families 4, 13, and 27 agree rather well with the ratio calculated, 15:1.

COMBINATIONS OF SUSCEPTIBILITY AND RESISTANCE OF F_3 FAMILIES TO FORM 15 AND FORM 27

The results obtained with form 15 and form 27 for those families which were inoculated with both forms are correlated in table 8. Various com-

TABLE 8.—*The classified reaction of individual F_3 families of Federation × Khapli to forms 15 and 27 of P. graminis tritici*

Reaction to form 27	Reaction to form 15					Total no. of families
	R	HR	H	HS	S	
R						0
HR		35[a], 39	16 32, 34	1, 10		7
H		9, 26	5, 7, 8, 11 18, 22 27, 29, 30	2, 4, 13, 28, 33		16
HS			21, 41	3, 12		4
S				23		1
Total no. of families		4	14	10	0	28

[a] Each number represents the F_3 family number. Families with less than eight individuals are underlined.

binations resulted. No families appeared, however, which were homozygous for susceptibility or resistance to both forms. There were two families

which appeared heterozygous in their reaction to the two forms, with susceptibility dominant. One family reacted as heterozygous to both forms, with resistance dominant. Many families fell into the H class when their reaction to forms 15 and 27 was correlated. Some were heterozygous to one form and heterozygous, with susceptibility dominant, to the other form. One family appeared heterozygous to form 15 and heterozygous, with resistance dominant, to form 27.

Families 1 and 10 were the most promising from the agronomic standpoint. Each of these was classified as heterozygous, with susceptibility dominant, to form 15; and heterozygous, with resistance dominant, to form 27. In both families the plants consisted of vulgare, intermediate, emmer, and durum types but the intermediate type predominated.

THE F_3 PLANT TYPES

A portion of the grain harvested from each F_2 plant was sown in the field at University Farm, St. Paul, in the spring of 1924. Some of the grains failed to germinate while others grew to the "tuft" stage and then died off. From the sowing 371 plants were harvested. These were of various types and showed wide differences in percentage fertility. Of the

TABLE 9.—*Numbers of F_3 plants of Federation × Khapli of different fertility percentages in each of the four wheat classes*

Percentage fertility	Vulgare	Intermediate	Emmer	Durum
0	4	8	28	22
1–5	1	3	8	7
6–10	–	5	9	11
11–15	2	4	8	8
16–20	2	7	7	–
21–25	5	7	4	5
26–30	2	3	7	3
31–35	1	7	3	4
36–40	4	11	5	6
41–45	1	19	9	6
46–50	3	13	4	2
51–55	3	11	3	4
56–60	9	19	2	–
61–65	2	12	1	1
66–70	3	3	3	3
71–75	1	8	–	1
76–80	1	1	–	–
81–85	–	2	–	–
Totals	44	143	101	83

total number of plants, 71 were dwarfs and the remainder of normal type. These dwarfs varied from 8 to 12 inches in height. Some were quite sterile, while in others about 30 per cent of the spikelets produced grain.

A detailed study was made of the morphological characters of each plant and the types classified from the data obtained on the density[3] of the heads, prominence of keel, and percentage of grain which thrashed free of the glumes after being put once through an individual plant thrasher. The percentage fertility was determined from counts made on the number of florets and number of grains set per spike. All determinations made were based on the examination of two representative heads from each plant. The individual plant data which formed the bases of classification is not presented here. It is of interest, however, to know the number of individuals which fell into the various classes and the fertility of each. This information is given in table 9.

Of the 30 F_2 plants grown in the F_3 generation, 15 were intermediates, 7 were durums, 6 were emmers, and 2 were vulgare-like. The progeny of five of the F_2 durum plants bred true for characters of the durum class, though they were not absolutely fixed. Most of the progeny of the F_2 intermediates consisted of types belonging to the emmer, durum, vulgare, and intermediate classes, while the F_2 emmer plants produced progeny of emmer and durum types. A few of the F_2 durum plants gave progeny in the F_3 which consisted of durum and emmer types. No attempt was made to differentiate between the emmer and spelt types. These types were classified together. A study of the figures for density of head showed that both lax and dense types were to be found in the three classes, emmer, durum, and vulgare. The character of the keel on the outer glume and the percentage of grain which had adherent glumes after thrashing, however, served to differentiate these three types.

REACTION OF F_4 FAMILIES TO STEM RUST IN THE FIELD

About 125 F_3 plants which were of intermediate or vulgare type, and which produced good quality grain, were selected to test the reaction of the progeny to stem rust in the field.[4] These tests were carried out in the rust nursery, University Farm, St. Paul, in the summer of 1925, when the seasonal conditions were particularly favorable for the development of rust. Thirteen physiologic forms of *P. graminis tritici* were used to produce the epidemic.

[3] Density of head was calculated by dividing the length of the head in millimeters by the number of spikelets less one.

[4] Twenty-five to thirty grains of each F_3 plant sown.

All of the hybrid material was susceptible, or of a semi-resistant nature which closely approached susceptibility. Some lines, however, appeared to be segregating for a susceptible and semi-resistant type of reaction. The percentage rust development on the hybrids varied from 65 to 100, although one line which was from an intermediate plant appeared semi-resistant and had 50 per cent infection. The Federation parent had on the average 100 per cent rust, while Khapli averaged 5 per cent in the development of rust. The progeny of a number of F_3 emmer and durum plants were also included in the test, and these proved to be quite susceptible. There appeared to be no relation between head type and rust reaction. The results of the F_4 rust tests are presented in table 10.

TABLE 10.—*The reaction of certain F_4 families of Federation × Khapli to thirteen physiologic forms of stem rust in the Rust Nursery, University Farm, St. Paul, showing the number of families in the different wheat classes*[a]

Percentage stem rust		Number of F_4 families			
		Vulgare	Intermediate	Emmer	Durum
50	SR[b]		1		
65	S[c]			1	
	SR		3		
75	S		2	5	
	SR	2	8		
80	S			2	
	SR		2		
85	S	1			
	SR	1	1		
90	S	7	7	7	
	SR	3	8	1	1
95	S		12	1	1
	SR		9		
100	S	24	32	52	6
Segregating			4		

[a] The classification is that of the F_3 parent plants.
[b] SR = semi-resistant.
[c] S = susceptible.

These results serve to emphasize the difficulty of obtaining from species crosses, wheat varieties which are of common type and which are resistant to several forms of rust. Further work is in progress dealing with the reac-

tion of certain F_5 families to the two physiologic forms of stem rust which are present in Australia (18).

SUMMARY

1. In 1921 a successful cross was made between Federation wheat (*Triticum vulgare*) and Khapli emmer (*T. dicoccum*). This was done at Sydney University, New South Wales, Australia.

2. The F_1 generation consisted of partially sterile intermediate types. In the F_2 many different types appeared which showed in varying degrees the characters of the vulgare, emmer, durum, and intermediate groups. Variation occurred between complete fertility and complete sterility.

3. A study was made of the parasitic capabilities of two physiologic forms of *Puccinia graminis tritici* on the F_3 hybrids under glasshouse conditions.

4. Forms 15 and 27 were selected for the tests. These were known to be constant in their parasitic behavior. Federation, C. I. 4734, was susceptible to both forms, and Khapli, C. I. 4013, resistant to both.

5. Thirty F_3 families were inoculated with form 15 when about three inches high. After the infection types were recorded, the first leaf of each plant was removed and the second leaf inoculated with form 27. In analyzing results families with less than eight individuals were disregarded.

6. Some F_3 families showed all gradations between complete resistance and complete susceptibility to both forms of rust. With both forms there appeared all combinations of size of uredinia and degree of hypersensitiveness.

7. The inheritance of resistance and susceptibility in the F_3 generation to forms 15 and 27 may be interpreted on a multiple factor hypothesis.

8. One family appeared homozygous for susceptibility to form 27 although no families were homozygous for resistance to either of the forms. Some families were heterozygous, with resistance or susceptibility dominant; others were distinctly heterozygous, with plants evenly distributed in the resistant, intermediate, and susceptible classes.

9. Combinations of susceptibility and resistance of individual F_3 families to the two physiologic forms were studied. One family was heterozygous in its reaction to both forms, with resistance dominant.

10. Part of the progeny of thirty F_2 plants was grown in the field at University Farm, St. Paul. Of a total of 371 plants harvested, 71 were dwarfs and the remainder of normal type. These plants were classified into emmer, durum, vulgare and intermediate groups on the bases of prominence of keel, density of heads, and the percentage of grain which had adherent glumes after threshing. Some of the F_2 durum plants' progeny bred true for characters of the durum class, while most of the F_2 intermediate and

emmer types segregated in the F_3 generation. All variations in fertility occurred between 0 and 85 per cent.

11. The progeny of over 200 F_3 plants from the vulgare, intermediate, emmer, and durum classes was tested in the rust nursery for reaction to 13 forms of stem rust. Most of the families proved to be susceptible, but some were segregating for a susceptible and semi-resistant type of reaction. The rust development on the hybrids varied from 50 to 100 per cent. The reaction of most families was a susceptible one although some were semi-resistant. There appeared to be no relation between head type and rust reaction.

12. The reaction of certain F_5 families to the two forms of stem rust in Australia is now under investigation.

In conclusion the writer desires to acknowledge his indebtedness to Professors E. C. Stakman and H. K. Hayes, of the University of Minnesota, for helpful advice and criticism, and also to Mr. O. S. Aamodt, of the United States Department of Agriculture, for recording infection notes on the hybrid material grown in the rust nursery, University Farm, St. Paul.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
ST. PAUL, MINNESOTA.

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EXPLANATION OF PLATES

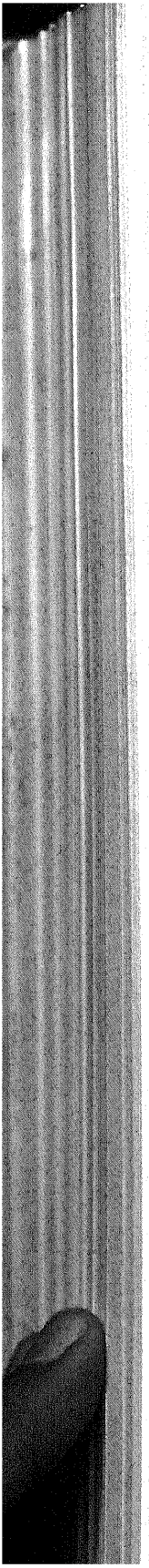
PLATES XLVIII AND XLIX

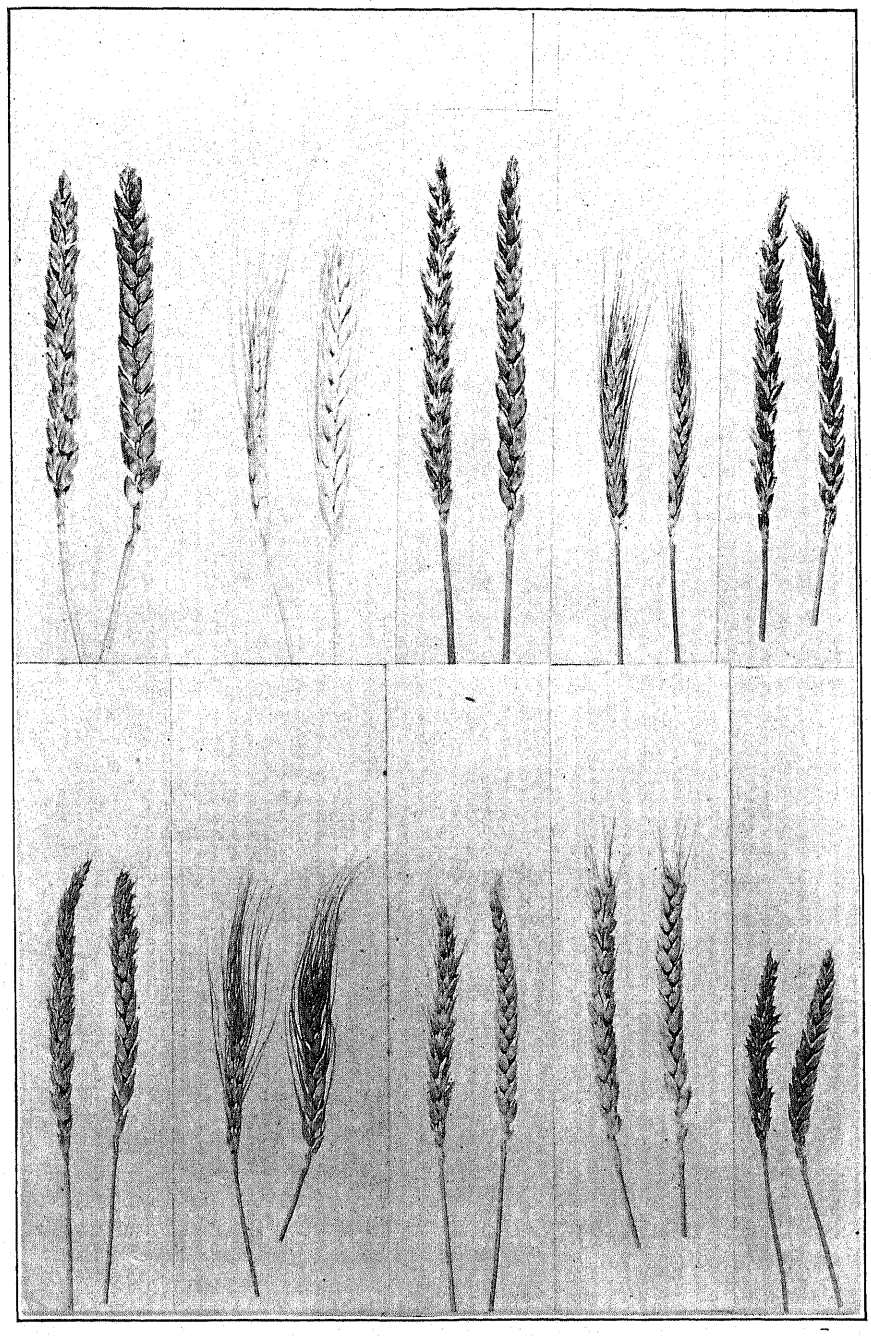
Representative types showing face and side views of F_2 plants of a cross between Federation wheat and Khapli emmer. The first two pairs of heads in the top section of Plate XLVIII are from typical plants of the parent varieties.

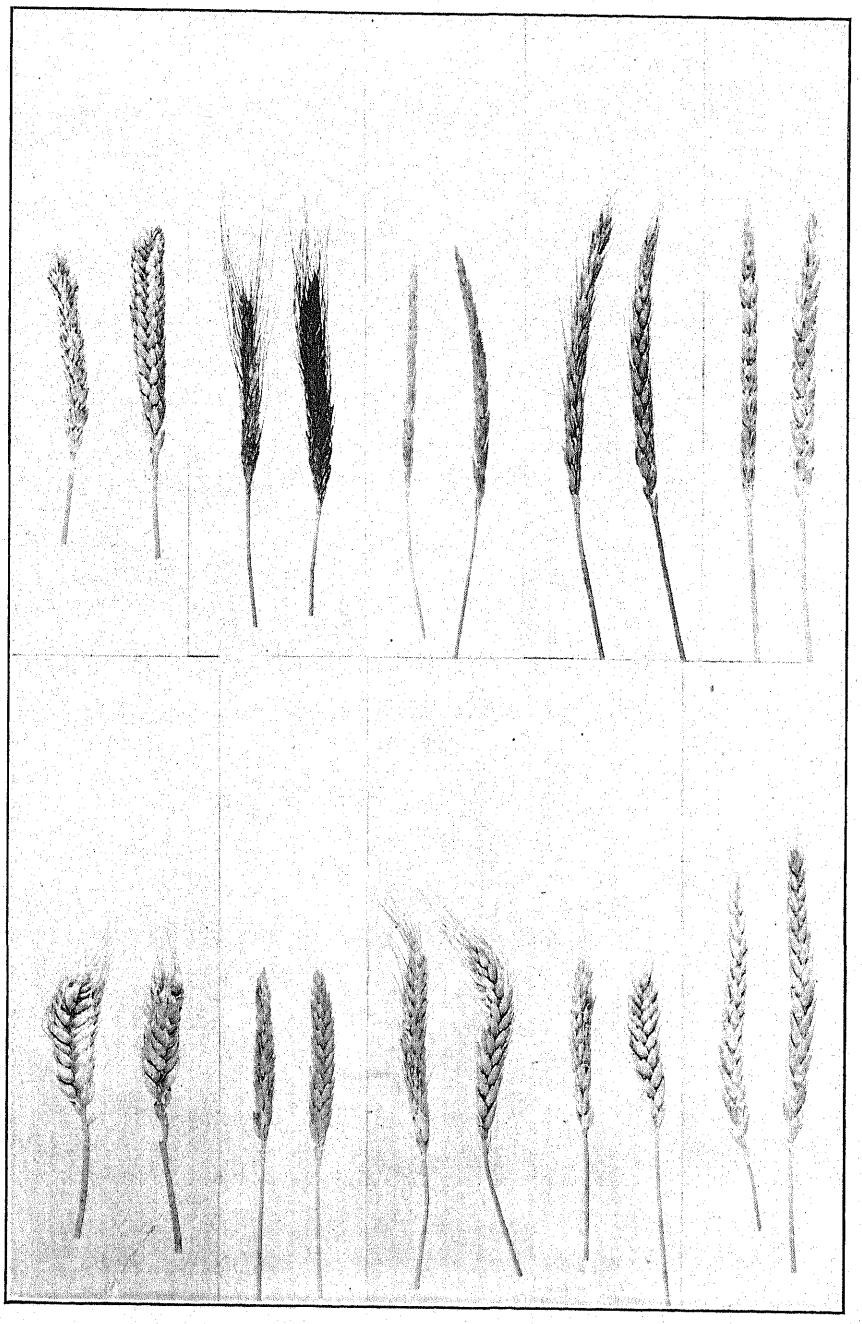
PLATES L AND LI

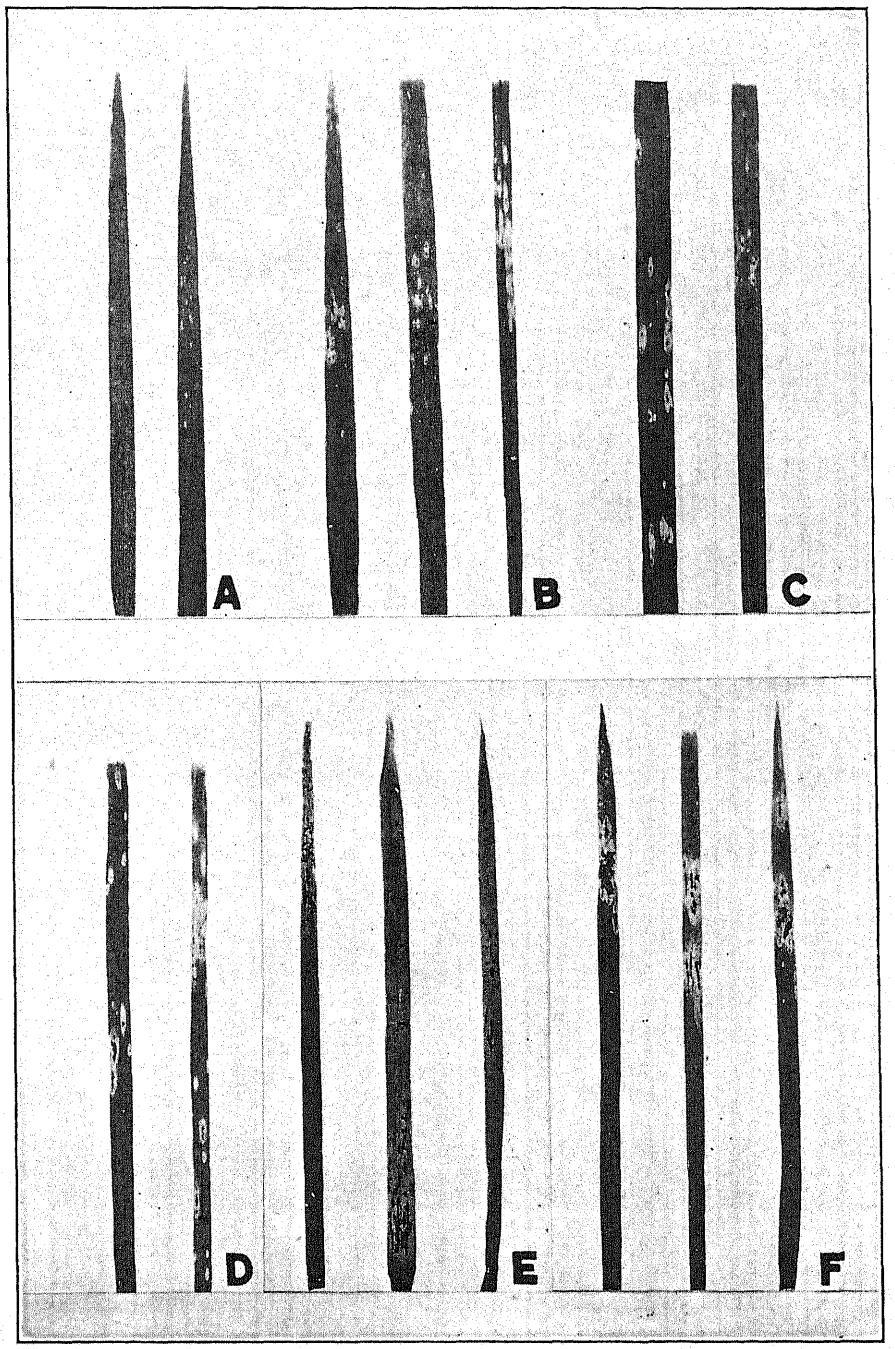
Reaction of F_3 plants of Federation × Khapli and the parent varieties showing types of infection obtained with *Puccinia graminis tritici* form 27:—

A. 0;
B. 1
C. 2
D. X
E. 3
F. 3^n
G. 4
H. 4^n
I. 1 (Khapli)
J. 4^{n+} (Federation)





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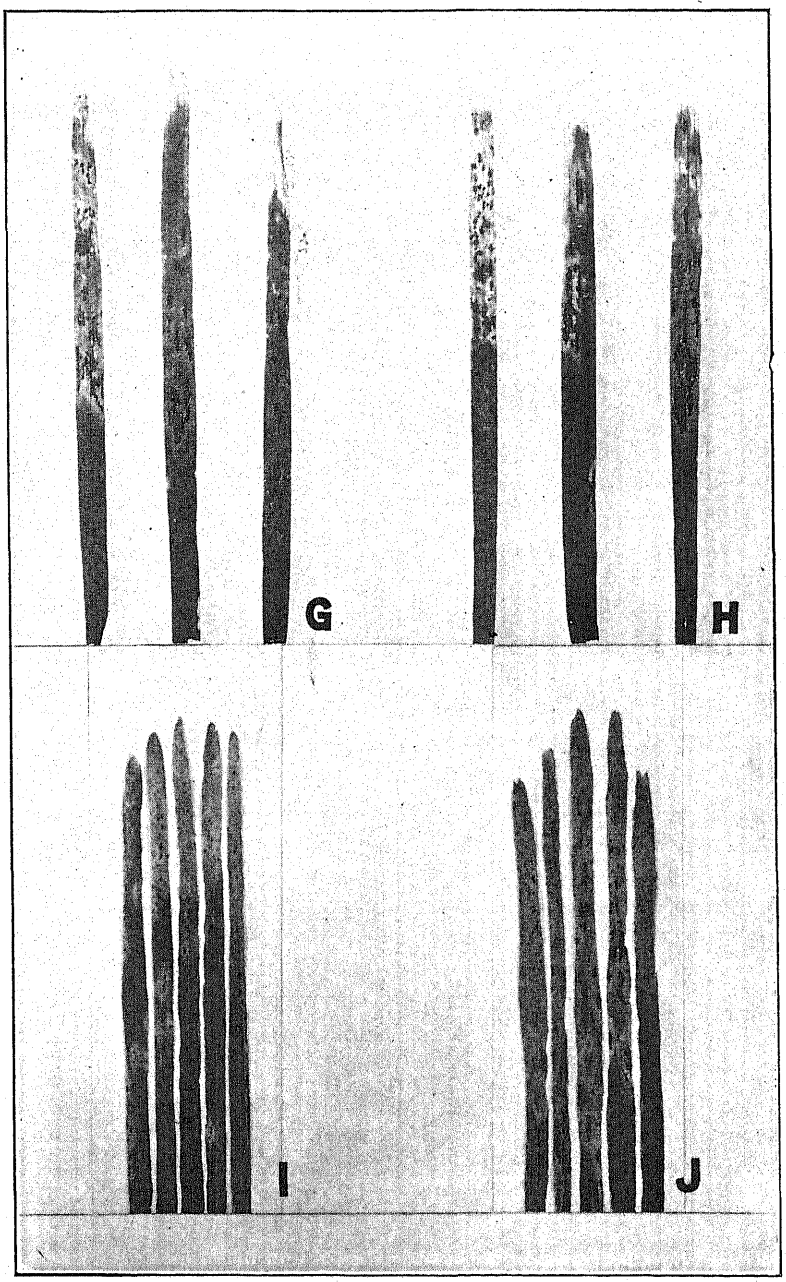
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SEED TREATMENTS FOR THE CONTROL OF BUNT OF WHEAT[1]

FRED N. BRIGGS[2]

INTRODUCTION

The popularity of the copper carbonate dust treatment for the control of bunt (*Tilletia tritici* [Bjerk.] Winter) in wheat has induced a number of chemical companies and individuals to undertake the production of other chemical dusts to be used for the control of seed-borne diseases, especially those of cereals. At about the time the value of the copper carbonate dust treatment was established in this country a number of organic mercury compounds were introduced for experimental use. From time to time new fungicides, both dust and liquid, have been received and tested for the control of bunt at Davis, California, during one or more years. The data are necessarily fragmentary because all of the fungicides were not available during the entire period and others were dropped after one year because they proved ineffective.

HISTORY

The literature dealing with seed treatment for the control of bunt has become so extensive that no attempt will be made to review all of it in this paper. As early as 1902 Von Tubeuf (9) controlled bunt successfully with copper carbonate dust made in his own laboratory. Apparently no practical use was made of copper carbonate for the control of bunt as a result of Von Tubeuf's experiments. In 1904 Bolley (1) reported an unsuccessful attempt to find a satisfactory dust treatment for the control of bunt. Also, the same year, Wheeler (10) reported that ozonit gas powder, with which he had experimented, would not control bunt. In 1915 Darnell-Smith (2) reported satisfactory control of bunt with copper carbonate, and Mackie and Briggs (4) introduced copper carbonate into the United States for bunt control in 1920. Since that time it has become the most popular treatment

[1] Cooperative investigation between the Bureau of Plant Industry, United States Department of Agriculture, and the Agricultural Experiment Station of the University of California. Throughout the entire progress of this investigation the writer has received many helpful suggestions from Professor W. W. Mackie of the Division of Agronomy, University of California, and from Dr. W. H. Tisdale of the Office of Cereal Investigations.

[2] Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

TABLE 1.—*The efficiency of various liquid fungicides for the control of bunt of White Federation wheat at Davis, California*

Treatment	Per cent of Smutty Heads											
	1922 Crop			1923 Crop			1924 Crop			1925 Crop		
	Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
Check												
Untreated	47.8	38.4	20.4	55.7	35.3	17.0	61.1	53.1	37.3	63.6	57.3	39.7
Bluestone (1–5)3 min.	.6	0.0	.4	0.0	.5	0.0	.4	0.0	0.0	0.0	0.0	0.0
Bluestone (1–4)3 min.—Lime (1–8)5 min.	1.4	.4	.6	0.0	.3	3.8	6.5	2.1	1.0	4.6	1.8	1.1
Formaldehyde (1–40) 10 min.	.3	.1	0.0	0.0	.9	.6	0.0	0.0	0.0	0.0	0.0	0.5
Solutions												
Chlorophol .1% 30 min.				.6	.4	.3	8.7	4.0	1.1			
.2% do				.6	.2	0.0	1.8	2.9	.2			
.3% do				0.0	.5	0.0						
.1% 1 hour				3.0	.5	.4						
.2% do				.3	0.0	.4						
.3% do	.6	0.0	0.0	.8	0.0	0.0						
Corona No. 620, .05% 30 min.				3.1	2.7	.7						
.1 % do				2.0	0.0	1.0	8.9	4.6	1.4			
.2 % do				1.0	.2	0.0	5.7	.7	1.0			
.05% 1 hour				2.0	.6	1.8						
.1 % do				2.2	.7	.3						
.2 % do				.5	1.0	.5						
Semesan .1% 30 min.				7.0	1.9	1.1						
.2% do				.6	0.0	.5						
.3% do				.2	.3	.5						
.1% 1 hour				2.5	.3	1.2						
.2% do				.2	0.0	0.0						
.3% do				.4	.4	.1						

TABLE 1.—(*Continued*)

	Per cent of Smutty Heads											
	1922 Crop			1923 Crop			1924 Crop			1925 Crop		
	Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
Germisan .1% 30 min.				0.0	0.0	.1	14.3	6.5	1.8			
.2% do				0.0	.5	0.0	3.1	.9	0.0			
.3% do				0.0	0.0	0.0						
.1% 1 hour				0.0	0.0	0.0						
.2% do				0.0	0.0	0.0						
.3% do				0.0	0.0	0.0						
Dupont No. 12, .1% 30 min.				1.7	1.0	.8	8.0	3.7	2.1			
.2% do				0.0	0.0	0.0	3.4	1.4	1.1			
Dupont No. 12, .1% 1 hour				1.4	0.0	0.0						
.2% do				.3	.7	.1						
Uspulun .1% 30 min.							14.6	9.0	3.2			
.2% do							4.2	1.5	.7			
Furfural .1% 30 min.							51.0	48.2	30.9			
.2% do							61.2	48.3	36.3			

for the control of bunt in this country because of its effectiveness as a fungicide, lack of seed injury, and ease of application and low labor cost.

Tisdale et al (8) have recently given an account of the development of chlorphenol mercury as a bunt fungicide. It is the basis for such commercial preparations as Uspulun, Chlorophol, and Semesan. Riehm (7) and Remy and Vasters (6) were pioneers in the development of the chlorphenol mercury treatments.

METHODS

A pure line White Federation wheat was used in these experiments because it is a bunt-susceptible wheat and because the smut counts are relatively easy to make in a white-chaffed variety. Three lots of seed were inoculated with spores of bunt, *Tilletia tritici*, with the following proportion of bunt spores to seed by weight: 1:250, 1:750, and 1:1500. The three rates of inoculation were used to secure a better indication of the range of effectiveness of a given fungicide than could be obtained with a single lot.

Duplicate sowings were made in rod rows which were spaced 12 inches apart. One hundred and fifty seeds were sown in each row.

At harvest time the plants were pulled and the total number recorded in order to get an idea as to the effect of the various treatments on the stands of grain. The total number of heads and the smutted heads were recorded and the percentage of smut calculated from these data. A partially smutted head was considered as smutted.

Check rows of untreated seed and seed treated with bluestone, bluestone-lime, formaldehyde, and copper carbonate were included for comparison.

RESULTS WITH SOLUTIONS

Comparatively few solutions were investigated because the farmer prefers a dust treatment, other things being equal. The results obtained with seven different liquid fungicides are recorded in table 1.

The percentage of bunt in the untreated rows was greater in 1924 and 1925 than in the two preceding years. This was due no doubt to climatic conditions at the time of planting. The bunt infection is high enough in each case to give a good measure of the effectiveness of the various treatments. Formaldehyde controlled bunt better than bluestone and bluestone better than bluestone-lime. Absolute control is the goal in seed treatment but many treatments which may be considered entirely satisfactory will not completely eliminate the smut where the seeds are inoculated as heavily as they were in these experiments.

Furfural is the only one of the solutions that did not give satisfactory control and may be dropped from further consideration. A .1 per cent solu-

tion of Chlorophol produced good results in 1923, but in 1924 with a heavier bunt infection the .2 per cent solution produced better results. With Corona No. 620 the results were not quite so good, yet they may be considered satisfactory. A .2 per cent solution of Semesan gave better results than a .1 per cent solution. In 1923 Germisan was outstanding as a fungicide, but the comparatively poor results obtained in 1924 with material from the same supply suggest that it may lose some of its effectiveness on standing. Dupont No. 12 ranks along with Chlorophol and Corona No. 620, while Uspulun is somewhat less effective, being about equal to Germisan in 1924.

The amount of seed injury caused by a given fungicide is very important and is a point which has been given much consideration only during the last few years. In these experiments 150 seeds were sown in each row, and the number of plants present at harvest time was recorded in order to determine the comparative seed injury in each case. While all the plants emerging may not live to maturity, more exact counts may be made at the later date with greater facility. Since the number of plants present at harvest time in each treatment (Table 2) is an average of six rows, it is believed that the comparative figures are reliable.

Bluestone was very injurious to germination in 1922 and 1925. The injury was not so great in the other two years, but the number of plants from treated seed was significantly lower than that from untreated seed. Formaldehyde ranked next to bluestone from the standpoint of seed injury. However, in 1923, formaldehyde caused no injury. The reasons for these seasonal fluctuations with the bluestone and formaldehyde treatments are not well understood. The lime dip following the bluestone treatment prevented most of the seed injury.

Lack of seed injury is oustanding with all of the other solutions used. In 1923 all of the differences were well within three times the probable error as compared with the untreated. Some of the probable errors are very high because the field was infested in spots with morning glory. Seeds treated with Chlorophol, especially the .2 per cent solution for one hour, produced the greatest number of plants. Differences between the other solutions are small and are not significant.

In 1924 seed treated with the five solutions tested produced higher percentages of mature plants than did the untreated seed. The reasons for the greater differences in 1924 than in 1923 are not apparent. The condition of the seed, the soil, the climate, or other factors may be concerned.

Although these new fungicides, except furfural, caused satisfactory bunt control and did not cause any seed injury, in all probability they will not be used for the control of bunt on the West Coast because of the

TABLE 2.—*The number of White Federation wheat plants at harvest from 150 seeds untreated or treated with various fungicides. Average of six seedings at Davis, California*

Treatment	Number of plants at harvest			
	1922	1923	1924	1925
Checks				
Untreated	86.7 ± 1.17	107.2 ± 5.99	95.0 ± 1.08	105.7 ± 1.90
Bluestone (1–5)3 min.	45.8 ± 0.73	82.5 ± 4.86	85.7 ± 2.86	40.0 ± 2.13
Bluestone (1–4)3 min.—Lime (1–8)5 min.	71.8 ± 1.23	105.2 ± 6.25	96.2 ± 1.40	94.3 ± 2.33
Formaldehyde (1–40)10 min.	59.0 ± 1.79	102.0 ± 4.16	58.0 ± 2.89	63.5 ± 0.61
Solutions				
Chlorophol .1% 30 min.		113.3 ± 4.88	119.0 ± 2.80	
.2% do		110.5 ± 4.65	115.3 ± 2.70	
.3% do		105.0 ± 6.14		
.1% 1 hour		109.3 ± 3.80		
.2% do		118.3 ± 1.74		
.3% do	86.6 ± 4.67	112.5 ± 3.09		
Corona No. 620 .05% 30 min.		107.5 ± 2.86	112.5 ± 2.15	
.1 % do		111.5 ± 1.59	116.0 ± 1.35	
.2 % do		100.8 ± 2.86		
.05% 1 hour		106.3 ± 3.80		
.1 % do		104.3 ± 1.93		
.2 % do		98.8 ± 3.51		
Semesan .1% 30 min.		102.3 ± 5.26		
.2% do		103.5 ± 3.02		
.3% do		106.8 ± 5.15		
.1% 1 hour		105.5 ± 5.85		
.2% do		107.3 ± 2.75		
.3% do		98.0 ± 5.58		
Germisan .1% 30 min.		100.8 ± 5.53	114.7 ± 2.90	
.2% do		103.2 ± 4.96	113.3 ± 2.20	
.3% do		103.3 ± 3.14		
.1% 1 hour		105.8 ± 2.78		
.2% do		107.2 ± 1.38		
.3% do		101.7 ± 2.43		
Dupont No. 12 .1% 30 min.		105.8 ± 3.19	120.2 ± 2.64	
.2% do		102.5 ± 3.24	117.3 ± 1.95	
.1% 1 hour		106.5 ± 3.98		
.2% do		106.6 ± 5.44		
Uspulun .1% 30 min.			117.8 ± 1.72	
.2% do			119.8 ± 1.87	

greater ease of application of the dust treatments and the low cost of copper carbonate.

DUSTS

Twenty-four fungicidal dusts other than copper carbonate were investigated in the four-year period. During the first two years the dusts were applied at the rate of from 1 to 4 ounces per bushel. Later the 4-ounce treatment was dropped because with copper carbonate this rate of application was not superior to the 3-ounce treatment and because it was not possible to get this amount of dust to adhere to the seed. The effects of the various dusts on bunt control are recorded in table 3.

The dusts which did not approach copper carbonate in effectiveness and which may be dropped as unsatisfactory are: Seed-O-San, S. D. No. 4, S. D. No. 6, S. D. No. 7, 40–F, 40–S, C-10-F, C-10-G, C-20-F, C-20-G, Karasch compounds A and B, Dupont 8 and 9, Copper Stearate, and Dupont No. 19 A and No. 19 B. Copper carbonate is used as a basis for comparison because it is the only dust treatment that has been extensively used for bunt control and because it has given satisfactory results in the hands of the farmer. In order for a fungicide to replace copper carbonate, it should control bunt as well as or better than copper carbonate, it should be cheaper, and should give stands of plants equal to or better than the stands from seed treated with copper carbonate. Nothing is said about the increase in yield due to seed treatment because we believe that sufficient data are not yet available to warrant any statement.

Coppercarb, a copper compound with less than half the copper content of copper carbonate, produced fairly satisfactory results. However, it takes about twice as much dust per bushel under the conditions of these experiments to equal pure copper carbonate.

Chlorophol is a chlorphenol-mercury compound and was intended by the manufacturers to be used as a solution. However, it gave satisfactory results when used as a dust in 1922. It was not tested further because of its greater cost.

S. D. No. 3 controlled bunt fairly satisfactorily but was not as efficient as S. D. No. 5, which is the concentrated fungicide that forms the toxic principle of the Semesan used in 1925. Semesan and Dupont No. 2 compare favorably with copper carbonate for the control of bunt.

In summarizing the results the data indicate that Chlorophol, S. D. No. 3 and S. D. No. 5, Dupont No. 2 and Semesan controlled bunt approximately as well as copper carbonate dust. S. D. No. 5 gave slightly superior control, especially at the rate of one ounce per bushel.

TABLE 3.—*The efficiency of various dust fungicides for the control of bunt of White Federation wheat at Davis, California*

Treatment		Per cent of Smutty Heads											
		1922 Crop			1923 Crop			1924 Crop			1925 Crop		
		Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
Dusts	Oz. per bu.	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
Check (untreated)		47.8	38.4	20.4	55.7	35.3	17.0	61.1	53.1	37.3	63.6	57.3	39.7
Copper carbonate	1	22.7	2.2	0.1	12.2	1.5	0.0	17.9	3.5	0.7	32.7	6.8	1.7
	2	9.4	1.2	0.0	4.5	1.3	0.7	5.0	4.1	0.7	13.9	3.0	0.4
	3	1.1	0.4	0.2	0.0	0.0	0.0	4.7	1.9	0.4	4.3	1.2	0.2
	4	2.3	0.1	0.0	0.8	0.0	0.0						
Coppercarb	1				12.3	1.5	2.3	21.6	10.4	2.7			
	2	16.1	5.1	2.5	8.3	2.7	0.3	13.0	6.7	2.6			
	3				7.8	0.1	0.1	8.1	4.6	2.5			
	4	9.9	0.0	0.4	3.6	2.2	0.0						
Chlorophol Dust	1												
	2	19.4	2.4	2.2									
	3												
	4	3.3	0.1	1.6									
Seed-O-San	1				39.9	19.7	11.0						
	2				38.9	6.1	4.6						
	3				32.5	5.5	1.6						
	4				14.6	1.2	0.0						
S. D. No. 3	1				7.5	2.3	0.6	15.4	5.8	1.8			
	2				2.8	0.5	0.0	8.6	1.8	1.0			
	3				1.9	0.5	0.5	4.2	1.3	1.1			
	4				0.7	0.0	0.0						

TABLE 3.—(*Continued*)

Treatment		Per cent of Smutty Heads											
		1922 Crop			1923 Crop			1924 Crop			1925 Crop		
		Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
Dusts	Oz. per bu.	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
S. D. No. 4	1				25.9	5.8	0.4						
	2				22.0	5.0	0.0						
	3				5.5	0.5	0.2						
	4				4.6	0.9	0.3						
S. D. No. 5	1				1.3	0.9	0.2	3.0	0.6	1.2			
	2				0.3	0.6	0.0	0.8	0.6	0.2			
	3				0.8	0.0	0.0	0.0	1.1	0.0			
	4				0.5	0.0	0.0						
S. D. No. 6	1				19.0	9.1	7.9						
	2				9.9	7.6	1.2						
	3				3.4	1.5	0.3						
	4				4.5	2.6	0.0						
S. D. No. 7	1				26.7	7.1	4.3						
	2				13.0	4.4	0.2						
	3				5.7	1.0	0.0						
	4				1.8	0.4	0.2						
40–F	1				52.7	22.4	7.0						
	2				33.0	15.4	2.3						
	3				26.9	5.5	0.3						
	4				14.3	2.5	1.1						
40–S	1				40.7	32.7	8.7						
	2				19.2	11.2	1.5						
	3				14.4	3.0	1.4						
	4				9.6	5.4	0.2						

TABLE 3.—(*Continued*)

Treatment		Per cent of Smutty Heads											
		1922 Crop			1923 Crop			1924 Crop			1925 Crop		
		Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
Dusts	Oz. per bu.	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
C–10–F	1				46.5	20.7	11.1						
	2				46.4	26.8	7.0						
	3				45.9	20.5	11.1						
	4				35.3	17.7	5.6						
C–10–G	1				35.3	26.7	8.5						
	2				46.9	25.2	8.3						
	3				58.2	18.1	6.7						
	4				39.3	13.2	3.7						
C–20–F	1				41.4	19.2	5.0						
	2				35.9	8.6	1.8						
	3				28.5	8.9	2.6						
	4				9.0	2.8	4.2						
C–20–G	1				33.0	20.3	7.1						
	2				39.0	7.6	4.5						
	3				33.7	2.0	5.0						
	4				16.1	5.4	3.4						
Dupont No. 12	1				21.3	6.5	3.3						
	2				11.3	4.1	1.2						
	3				3.4	0.8	0.8						
	4				4.1	1.3	0.7						
Karasch compound A	1							64.7	50.4	29.4			
	2							58.8	47.6	29.6			
	3							52.6	40.6	24.7			

TABLE 3.—(*Continued*)

Treatment		Per cent of Smutty Heads											
		1922 Crop			1923 Crop			1924 Crop			1925 Crop		
		Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
Dusts	Oz. per bu.	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
Karasch compound B...	1							69.4	46.5	31.6			
	2							60.5	48.8	37.1			
	3							64.2	46.3	29.6			
Dupont No. 2	1							15.2	4.4	2.1	26.4	6.0	1.5
	2							7.0	2.6	2.2	14.0	3.1	1.8
	3							3.9	1.0	0.4	4.8	1.5	0.0
Dupont No. 8	1							52.0	29.7	10.7			
	2							38.0	17.2	6.7			
	3							22.3	16.0	4.1			
Dupont No. 9	1							63.4	25.4	20.2			
	2							49.6	22.1	8.4			
	3							33.9	16.3	2.7			
Copper stearate	1										56.9	31.6	17.0
	2										63.6	30.7	21.2
	3										52.5	32.8	16.4
Semesan	1										22.3	9.7	2.3
	2										10.7	1.2	2.1
	3										12.3	2.2	0.6
Dupont No. 19A	1										69.9	44.6	29.6
	2										65.0	38.1	22.1
	3										67.9	36.8	22.5
Dupont No. 19B	1										62.1	47.5	20.1
	2										56.6	38.1	25.2
	3										60.6	34.5	23.5

The number of mature plants derived from 150 seeds subjected to each of the various dust treatments gives a measure of seed injury, and is recorded in table 4.

TABLE 4. — *The number of White Federation wheat plants at harvest from 150 seeds treated with various dusts. Average of six seedings at Davis, California*

Treatment		Number of plants at harvest from 150 seeds			
Dust	Oz. per bu.	1922	1923	1924	1925
Checks (untreated)		86.7 ± 1.17	107.2 ± 5.99	95.0 ± 1.08	105.7 ± 1.90
Copper carbonate	1	83.0 ± 1.56	93.0 ± 2.45	97.1 ± 2.43	105.5 ± 1.51
	2	85.2 ± 1.79	97.8 ± 4.94	96.3 ± 1.43	109.0 ± 2.64
	3	85.2 ± 3.02	105.1 ± 4.56	101.5 ± 2.39	106.0 ± 1.72
	4	83.3 ± 2.39	100.8 ± 1.79		
Coppercarb	1		112.8 ± 2.41	99.7 ± 2.06	
	2	89.8 ± 1.20	111.1 ± 2.67	103.0 ± 2.32	
	3		110.0 ± 2.67	107.2 ± 0.67	
	4	89.0 ± 1.81	106.7 ± 2.86		
Chlorophol dust...	1				
	2	90.7 ± 2.29			
	3				
	4	88.7 ± 1.97			
S. D. No. 3.....	1		102.0 ± 1.03	113.2 ± 0.95	
	2		105.1 ± 2.77	118.5 ± 3.06	
	3		109.8 ± 4.72	116.7 ± 0.72	
	4		116.5 ± 3.66		
S. D. No. 5.....	1		106.3 ± 1.93	118.8 ± 2.35	
	2		100.1 ± 4.05	120.2 ± 2.89	
	3		107.8 ± 4.20	128.8 ± 1.72	
	4		109.0 ± 4.61		
Dupont No. 2.....	1			105.3 ± 2.46	108.7 ± 1.13
	2			112.2 ± 0.99	108.3 ± 2.27
	3			110.2 ± 2.22	105.0 ± 2.39
Semesan	1				107.5 ± 1.30
	2				112.3 ± 1.07
	3				110.6 ± 0.82

In comparing the results of the various treatments with those from untreated seed, it will be seen that there are no significant differences in 1922 and 1923. In 1924 all of the seed treated with S. D. No. 3 and No. 5 and Dupont No. 2 produced stands significantly better than the stands produced by untreated seed while there were no significant differences between

the results obtained from the copper carbonate and coppercarb dust treatments. In 1925, Semesan at the rate of two ounces per bushel was the only treatment superior to the untreated.

As two ounces of copper carbonate dust per bushel of wheat generally is recommended, the stands secured with the other dust treatments may be compared with those from this rate of application. In 1922 none of these treatments was significantly superior to copper carbonate dust in so far as improving stands is concerned. In 1923, S. D. No. 3 at the rate of four ounces produced better results. In 1924, all of the treatments with S. D. No. 3, S. D. No. 5, and Dupont No. 2, and the 3-ounce treatment with coppercarb were superior to copper carbonate applied at the rate of two ounces per bushel. In 1925, there were no significant differences. The reasons for the superior stands in 1924 when the organic mercury compounds were used are not known.

On the whole, the dust treatments caused no seed injury, as evidenced by stands from the treated seed as good as those from untreated seed. From this standpoint they are distinctly superior to the bluestone and formaldehyde treatments which were widely used for many years on the West Coast, especially the bluestone. In more recent years the injury from bluestone was largely prevented by the use of the lime dip. At present copper carbonate is widely used in place of the bluestone and bluestone-lime treatments, and has proven entirely satisfactory both from the standpoint of bunt control and lack of injury to the seed.

SUMMARY

The data presented herein on the control of bunt by liquid and dust treatments were collected over a four-year period beginning in 1922.

In most cases the more promising fungicides were investigated for two years.

All of the liquid fungicides, except furfural, controlled bunt satisfactorily.

Severe seed injury resulted from the use of bluestone. This injury was largely eliminated by the use of the subsequent lime dip. Formaldehyde caused severe injury except in 1923.

There was no injury from the use of Chlorophol, Corona No. 620, Semesan, Germisan, Dupont No. 12, and Uspulun. The stands obtained from seed treated with these compounds were not significantly different from the stands from untreated seed in 1923, but were better in 1924. The reasons for the better stands in 1924 are not known.

Twenty-four dust treatments other than copper carbonate dust were investigated during the four-year period. Of this number only six—Corona Dust, Chlorophol, S. D. No. 3, S. D. No. 5, Dupont No. 2, and Semesan—

controlled bunt as satisfactorily as did copper carbonate. S. D. No. 5 produced slightly better results than copper carbonate, especially with the one-ounce treatment.

Stands of wheat were improved in 1924 by the use of S. D. No. 3, S. D. No. 5, Dupont No. 2, and coppercarb (3 oz. per bu.), as compared with untreated seed and seed treated with two ounces of copper carbonate per bushel. In other years no significant differences were noted.

Liquid treatments are not likely to replace the dust treatments on the West Coast of the United States because of the greater ease in handling the latter.

Of the dusts used, all factors considered, copper carbonate is the most satisfactory.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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EFFECT OF MOSAIC ON CARBOHYDRATE AND NITROGEN CONTENT OF THE TOMATO PLANT[1]

P. H. Brewer, James B. Kendrick and Max W. Gardner[2]

In order to determine what influence different soil conditions might have upon the severity of the mosaic disease of the tomato, and also what effect the disease might exert upon the growth of the plant and upon its carbohydrate and nitrogen content, tests on potted plants were made in the greenhouse and supplemented by chemical analysis of the plants. Two types of mosaic were tested: typical tomato mosaic, and the necrotic or double-virus type (streak or winter blight).

In addition to one series of plants grown in a bench, two series of pot tests were carried out, one in the spring of 1924 and the other in the fall of 1925. Varnished metal pots of 10 kilograms capacity were used.

TESTS WITH STREAK OR DOUBLE-VIRUS MOSAIC

In the 1924 series, different soil conditions were provided by using a rich potting loam with high, medium, and low water content, and a clay loam very poor in nitrogen and phosphorus to which were added varying amounts of the lacking elements. On March 3, seedlings of the Bonny Best variety were planted in the pots. In this series the streak or necrotic (double-virus) type of mosaic was tested. Johnson (13, p. 10) and Dickson (6) have shown that this type of mosaic is due to the presence of more than one virus, namely, the virus of typical tomato mosaic or Fernow's (10) mosaic A, and a virus from potatoes which is probably the virus of the mosaic designated as B by Fernow (10). Inocula were obtained from two sources: (a) the streak or winter blight type of mosaic in a greenhouse crop of tomatoes, and (b), a necrotically mottled tomato fruit shipped from California to the Chicago market and sent to us by Dr. G. K. K. Link. This inoculum produced a necrotic type of mosaic less extreme than the preceding. For convenience these will be designated as the LaFayette (L) and California (C) types.

The inoculum was prepared by macerating the tissue in a flamed mortar and diluting with sterile water. Inoculation was effected by smearing the

[1] Joint contribution from the Botanical Department, Agricultural Experiment Station, and the Agronomy Department, Purdue University, Lafayette, Ind.

[2] The writers are indebted to Dr. E. G. Campbell and Mr. W. P. Allyn for assistance, and to Professor H. S. Jackson for helpful advice.

stem near the tip with a piece of absorbent cotton dipped in the inoculum and pricking through the smeared area with a flamed needle. On April 10 one plant in each series was inoculated with the LaFayette inoculum, one with the California inoculum, and one served as an uninoculated control.

On May 16, 36 days after inoculation, when the first fruits had set but before any fruits had approached maturity, certain of the plants were taken for fresh weight determination and chemical analysis.

In preparing the material for chemical analysis, the entire plant was weighed, chopped up, and placed in 95 per cent alcohol to which had been added calcium carbonate at the rate of one gram per liter. The pulp was later transferred to weighed beakers which were then placed on an electric hot plate and dried to constant weight. The percentage of dry matter was calculated from the difference in weight. The pulp was reduced to a fine powder and stored in tightly stoppered bottles until analyzed.

For the determination of sugars, a five gram portion of the dry powder was extracted with a 50 per cent alcohol solution in a Soxhlet extractor until all coloring matter had been removed. The solution was then made up to 250 cc. and the reducing sugars were determined by the picric acid colorimetric method (19) after an aliquot had been clarified by mercuric nitrate and sodium carbonate. The non-reducing sugars in this clarified liquid were inverted by the official hydrochloric acid method of inversion (7, p. 186) and determined by the picric acid colorimetric method (19). The polysaccharides in the residue in the extractor were hydrolyzed by the official hydrochloric acid method (7, p. 119) and determined by the picric acid colorimetric method (18). The nitrogen was determined by the Gunning-Kjeldahl method modified to include the nitrates (7, p. 8). The results of these analyses are shown in table 1.

The different soil conditions produced different growth responses as indicated by the differences in fresh weight and chemical composition of the healthy plants. But neither these differences nor differences in the soil moisture content greatly altered the character or severity of the mosaic symptoms on the inoculated plants.

However, there were outstanding and consistent differences between the mosaic and the healthy plants in each series. The mosaic plants were characterized in all cases by a reduction in total fresh weight, in percentage content of dry matter, in carbohydrate content, and in carbohydrate-nitrogen ratio. The reduction in weight of the roots was found to be proportional to the reduction in total weight. The reduction in carbohydrate content was due mainly to the lower content of polysaccharides.

The increase in total nitrogen shown by most of the mosaic plants was more apparent than real because of the lower percentage of dry matter in

TABLE 1.—*Effect of mosaic (streak type) on growth and on carbohydrate and nitrogen content of tomato plants*

Soil	Condition of plant	Fresh weight (grams)	Percentage dry matter	Percentage[1] reducing sugars	Percentage[1] non-reducing sugars	Percentage[1] polysaccharides	Percentage reduction in total carbohydrate content	Percentage[1] total N	Carbohydrate-nitrogen ratio
Rich potting soil	Healthy	657.1	12.7	1.19	1.94	26.8		3.76	7.9
	Mosaic C	419.0	11.4	1.25	1.88	13.5	44.4	3.72	4.5
	Mosaic L	288.0	10.0	1.10	1.70	13.0	47.0	4.36	3.6
P. C. L. + 500 N and 4000 P[2]	Healthy	455.7	14.3	1.25	2.50	38.4		1.45	29.1
	Mosaic C	432.1	12.8	1.18	1.97	37.7	3.1	1.85	22.1
	Mosaic L	241.2	12.8	1.10	2.20	26.8	28.7	2.16	13.9
P. C. L. + 500 N and 1000 P	Healthy	464.8	14.5	1.25	1.25	37.1		1.46	27.1
	Mosaic C	335.0	14.8	0.94	1.41	26.8	26.4	2.64	11.0
	Mosaic L	256.3	10.5	1.10	1.25	12.2	63.2	2.65	5.5
P. C. L. + 250 N and 4000 P	Healthy	360.4	14.7	0.94	1.26	38.4		1.39	29.2
	Mosaic C	274.1	11.8	0.94	0.94	32.9	14.3	1.63	21.4
P. C. L. + 250 N and 1000 P	Healthy	331.8	13.7	1.10	1.70	38.4		1.02	40.4
	Mosaic C	238.8	12.5	1.10	1.10	29.6	22.8	1.97	16.2
	Mosaic L	184.6	11.8	1.10	1.40	26.8	29.0	1.88	15.6

[1] Percentage based on dry weight.

[2] Poor clay loam plus 500 lbs. NH_4NO_3 and 4,000 lbs. 16 per cent acid phosphate per acre.

the mosaic plants. Even on the fresh weight basis, however, there was no evidence of a decrease in total nitrogen due to the disease.

In general, the more severe type of mosaic (L) caused the greater internal changes in the host. For example, the L type caused a reduction in total fresh weight of 44 to 56 per cent, the less severe C type, 5 to 36 per cent; the L type reduced the carbohydrate content 28 to 63 per cent, the C type 3 to 44 per cent.

In table 2 are shown the results of a bulk analysis of ten mosaic plants as compared with ten healthy ones of the Greater Baltimore variety grown in a greenhouse bench in June, 1925, and analyzed 23 days after inoculation. Seven of the inoculated plants showed only typical mosaic, and three the streak type. These plants were younger than those previously analyzed.

TABLE 2.—*Effect of mosaic on composition of young tomato plants*

Analytical tests	Healthy	Mosaic
Average fresh weight per plant	149.2 gm.	102.9 gm.
Dry matter	11.2 per cent	12.8 per cent
Reducing sugars	0.88 do	1.00 do
Non-reducing sugars	2.12 do	1.50 do
Polysaccharides	19.7 do	16.4 do
Total nitrogen	2.46 do	2.31 do
Carbohydrate-nitrogen ratio	9.23	8.18

The results in table 2 show that the mosaic plants were characterized by a reduction of 31 per cent in weight, 16.7 per cent in carbohydrate content, and 11 per cent. in the carbohydrate-nitrogen ratio. On the fresh weight basis there was no reduction in the nitrogen content.

TESTS WITH TYPICAL TOMATO MOSAIC

In the 1925 pot series, different soil conditions were provided by the addition of varying amounts of nitrogen and phosphorus to the poor clay loam, and of nitrogen, phosphorus, and potassium to Ottawa sand. Four pots of each type of soil were used. On October 29, 1925, a small plant of the Greater Baltimore variety (Indiana strain) was transplanted to each pot. On November 18, two of the plants in each series were inoculated with typical mosaic [type A of Fernow (10)], and the other two were left as uninoculated controls. On January 8, 51 days after inoculation, after the first fruits had set but before any had approached maturity, the two mosaic plants and two healthy plants from each series were taken for fresh weight determination and chemical analysis. The method of analysis varied only slightly from that previously outlined. The results are shown in tables 3 and 4.

TABLE 3.—*Effect of mosaic on growth and on carbohydrate and nitrogen content of tomato plants grown in poor clay loam with different fertilizer additions*

Fertilizer	Condition of plants	Average fresh weight in grams	Percentage[1] reducing sugars	Percentage[1] non-reducing sugars	Percentage[1] polysaccharides	Percentage reduction in total carbohydrate content	Percentage[1] total N	Carbohydrate-Nitrogen ratio
500 N + 1700 P[2]	Healthy	387	2.08	2.47	32.5		2.12	17.5
	Mosaic	328	1.14	1.99	20.5	36.2	2.52	9.4
500 N + 1200 P	Healthy	373	1.56	2.59	35.0		2.11	18.5
	Mosaic	330	0.94	1.96	21.0	38.9	2.31	10.3
500 N + 700 P	Healthy	311	1.25	1.88	28.0		2.16	14.4
	Mosaic	306	1.35	1.66	25.0	10.0	2.44	11.4
500 N + 200 P	Healthy	180	1.45	1.55	22.0		2.63	9.5
	Mosaic	192	1.00	1.50	19.0	14.0	2.28	9.4
125 N + 1200 P	Healthy	208	1.39	1.74	32.0		1.68	20.9
	Mosaic	170	1.17	1.34	28.1	12.8	1.42	21.5
125 N + 200 P	Healthy	129	1.25	1.45	29.0		1.96	16.2
	Mosaic	111	1.00	1.50	26.5	8.5	2.17	13.3

[1] Percentages based on dry weight.

[2] 500 lbs. NH_4NO_3 + 1,700 lbs. 20 per cent acid phosphate per acre.

TABLE 4.—*Effect of mosaic on growth and on carbohydrate and nitrogen content of tomato plants grown in Ottawa sand with different fertilizer additions*

Fertilizer	Condition of plants	Average fresh weight in grams	Percentage[1] reducing sugars	Percentage[1] non-reducing sugars	Percentage[1] polysaccharides	Percentage reduction in total carbohydrate content	Percentage[1] total N	Carbohydrate-Nitrogen ratio
500 N, 1500 P, 700 K, 400 Mg, 200 Fe[2]	Healthy	181	1.56	1.51	20.0		4.16	5.5
	Mosaic	134	0.78	1.52	19.0	7.6	3.59	6.0
500 N, 1500 P, 700 K	Healthy[3]	136	0.93	1.42	22.6		3.48	7.1
	Mosaic	160	1.10	1.19	21.5	4.6	3.55	6.7
100 N, 1500 P, 700 K. (Low N)	Healthy	55	1.11	1.97	36.7		2.06	19.3
	Mosaic	55	0.70	0.86	23.0	38.5	3.40	7.2
500 N, 75 P, 700 K. (Low P)	Healthy[3]	123	1.28	1.22	31.0		3.47	9.7
	Mosaic	25	0.75	1.45	26.7	13.7	3.95	7.3
500 N, 1500 P, 100 K. (Low K)	Healthy	94	0.78	1.22	35.0		4.56	8.1
	Mosaic	126	0.78	1.09	30.0	13.8	3.88	8.2
100 N, 75 P, 700 K. (Low N, P)	Healthy	37	0.90	1.30	36.3		1.83	21.0
	Mosaic	36	1.21	1.67	33.4	5.7	1.85	19.6
100 N, 1500 P, 100 K. (Low N, K)	Healthy	56	0.94	1.52	33.0		2.02	17.5
	Mosaic	46	0.88	1.62	25.0	22.4	2.26	12.1
500 N, 75 P, 100 K. (Low P, K)	Healthy	66	1.25	2.35	21.0		3.34	7.4
	Mosaic	75	0.85	1.65	20.5	6.5	4.20	5.5

[1] Percentage based on dry weight.

[2] 500 lbs. NH_4NO_3, 1500 lbs. $Ca_2(H_2PO_4)_2$, 700 lbs. K_2SO_4, 400 lbs. Mg_2SO_4, and 200 lbs. Fe_2SO_4 per acre. All pots received 200 lbs. $CaCO_3$ per acre.

[3] One plant used.

As in the 1924 series, there was no marked effect of different soil conditions upon the symptoms or severity of the disease, although these conditions did greatly affect the growth and composition of the plants. In general, the typical mosaic exerted in a somewhat milder degree the same effect upon the physiology of the host plant as did the double-virus type. In most cases the disease retarded growth as measured by fresh weight, and in all cases reduced the percentage content of carbohydrates. The nitrogen content was not consistently altered, and in most cases the carbohydrate-nitrogen ratio was lower in the mosaic plants.

DISCUSSION OF RESULTS

A reduced carbohydrate content and a normal nitrogen content in tomato mosaic are somewhat at variance with the phenomena found in similar diseases of certain other hosts. There was no marked decrease in total nitrogen as Jodidi, Kellogg, and True (11), and Jodidi, Moulton, and Markley (12) found in spinach mosaic, and hence no evidence that the denitrification theory advanced by Boncquet (2) and by Jodidi, Moulton, and Markley (12) will hold for the types of tomato mosaic studied. Likewise, the reduction of the carbohydrate content is the reverse of what happens in spinach mosaic as determined by True and Hawkins (21) and in potato leaf roll as determined quantitatively by Campbell (4), and lends no support to the theory that an abnormally high starch accumulation may be a characteristic of mosaic diseases in general.

In beet curly top, Bunzell (3) found little or no effect on the sugar or nitrogen content. In sugar cane mosaic, Lopez (16) found very little effect on the sugar content, but Asuncion (1) found 24 per cent less sugar in the diseased plants. Loew (15, p. 11) states that, in the case of tobacco, there is much less albumin in the mosaic leaves, and Woods (23, p. 13) states that the mosaic disease of tomato and tobacco is characterized by an accumulation of starch in the leaves, a view with which Tollenaar (20, p. 94) concurred.

W. P. Allyn found, in the course of work as yet unpublished (M. S. thesis, Purdue University, 1926), that starch constituted about 50 per cent of the polysaccharides in tomato plants and that there was a decrease in starch in the mosaic plants proportional to the decrease in polysaccharides. Furthermore, Allyn found that the mosaic diseases of cowpea and of soybean are also characterized by a reduction in the carbohydrate content.

Many of the earlier workers [Beijerinck, Koning (14, p. 69), Woods (22, p. 753)] believed that the mosaic disease of tobacco exerted an injurious effect upon the chloroplasts or the chlorophyll, and Dickson (5, p. 30) has shown that there is a decrease in number and a degeneration of the chloroplasts in the chlorotic areas accompanied by a decrease in carbohydrates.

Elmer (9, p. 81) has shown by quantitative tests that the mosaic disease of tomato greatly altered the composition of the chlorophyll. Sorokin (17) and Eckerson (8) have demonstrated cytologically the disintegration of the chloroplasts in tomato mosaic. Consequently, with these various disturbances of the apparatus involved in carbohydrate synthesis, it is not surprising that a reduced carbohydrate content was found in the diseased plants.

SUMMARY

1. Different soil conditions, as afforded by pot tests with fertilizers, did not greatly influence the character or severity of either of the two types of tomato mosaic tested, the streak or double-virus type and typical tomato mosaic.

2. In nearly all cases the mosaic plants were characterized by a reduction in total weight and, under all conditions, by a reduction in total carbohydrate content as shown by chemical analysis. This reduction occurred mainly in the polysaccharides.

3. There was, in general, no reduction in the nitrogen content and, as a rule, the carbohydrate-nitrogen ratio was lower in the diseased plants.

PURDUE UNIVERSITY,
LAFAYETTE, IND.

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STUDIES ON THE NATURE OF THE VIRUS OF TOBACCO MOSAIC[1]

MAURICE MULVANIA

INTRODUCTION

It has now been 34 years since Iwanowski (15) showed that the active principle in the production of the mosaic disease of tobacco would pass through a fine earthen filter. This was the beginning of the study of the so-called filterable viruses which recently has assumed so much importance both in plant and animal pathology. The nature of the causal agent responsible for tobacco mosaic has been the chief point of interest in practically all studies of this disorder, so far as the purely scientific aspect of the subject is concerned. The effort devoted to such investigations has been very great, yet so far as exact knowledge of the intimate nature of the virus is concerned it can not be said that very notable progress has been made. To be sure, these efforts have resulted in the accumulation of a large mass of facts as to the response of the virus to environmental conditions. These facts, however, are not sufficiently enlightening to permit the allocation of the virus either with non-living or living bodies: there is little agreement on this latter point among workers most intimately acquainted with the subject. In this direction attempted enlightenment seems to have enhanced the obscurity it was expected to displace.

The applicability of certain experimental procedures to the question of the nature of viruses appears to have been overlooked in some cases, and not to have been employed to the extent of their possibilities in this connection in other instances. Use has been made of some of these methods in the studies here reported; and it is hoped that the objective results secured may aid in the ultimate solution of the perplexing problem as to what viruses really are.

Experiments were conducted in five independent aspects of the general subject dealt with. These phases of the problem may be listed as follows:

1. Effect of light rays on the virus of tobacco mosaic
2. Effect of heat on the virus of tobacco mosaic
3. Effect of animal inoculation on the virus of tobacco mosaic

[1] The following studies were made under the direction of Dr. James Johnson, of the University of Wisconsin, to whom I am greatly indebted for his assistance and sustained interest in the work.

4. Dialyzability of the virus of tobacco mosaic

5. Effect of bacteria on the virus of tobacco mosaic. Each of these headings is considered as a separate unit in the body of the paper.

The general literature concerned with tobacco mosaic has been repeatedly reviewed by others and therefore will not be considered here except as it bears directly on the problem under discussion.

EFFECT OF LIGHT RAYS ON THE VIRUS OF TOBACCO MOSAIC

Raciborski (23) found that exposure of plant juice which contained the active principle of mosaic to sunlight for one day did not destroy the virus. Four or five days exposure was necessary to render it harmless to healthy plants when they were inoculated with it. His exposures were made in

TABLE 1.—*The effect of sunlight on the virus of tobacco mosaic*

Time of exposure in hours	No. plants inoculated	No. plants infected	Per cent of infection
None	10	10	100
¼	10	10	100
1	10	10	100
2	10	10	100
3	10	10	100
6	10	10	100
12	10	8	80
16	10	5	50
25	10	2	20
36	10	1	10

shallow dishes. The results are rather indefinite, and, as they constitute the only published data on this subject, should be verified and extended.

A number of detailed methods of exposing bacteria to the action of light have been described and used. Among these the very simple procedure of saturating threads with a culture of the organisms and then exposing the threads to the light appears quite as satisfactory as any, and this method was selected for use in our experiments on the action of daylight on the mosaic virus.

Fine silk threads were placed in the expressed juice of diseased plants and allowed to remain for 24 hours. They were then suspended from a cardboard in a position where the sunlight would fall directly upon them during the entire day. These threads were removed from the light according to a time schedule fixed upon, and placed in 1 cc. of water for 24 hours. The water in which the threads had thus been soaked was used as inoculum for young tobacco plants. No effort was made to prevent bacterial con-

tamination of the solutions, since the presence of such organisms could have no bearing on the development of the mosaic in the inoculated plants. The number of infections which resulted from the inoculations was taken as an index of the destructive action of light on the virus. Throughout the entire series of experiments this has been the criterion as to whether the virus had been affected by its subjection to the various treatments. The results secured by the sunlight experiments are shown in table 1.

Exposures of the virus to the action of ultra-violet rays were made in petri dishes. Berkfeld F candle filtrates from diseased tobacco plants were placed in the dishes to a depth of 1/8 inch. The uncovered dishes were then put immediately under a Cooper-Hewett mercury vapor lamp at a distance

TABLE 2.—*The effect of ultra-violet light on the virus of tobacco mosaic*

Time of exposure in hours	No. plants inoculated	No. plants infected	Per cent of infection
Series 1			
None	10	10	100
1/8	10	5	50
1/2	10	6	60
1	10	0	0
2	10	0	0
4	10	0	0
Series 2			
None	10	10	100
1/2	10	6	60
3/4	10	2	20
1	10	0	0

of six inches from the source of the rays. Observations of the temperature of the exposed solution showed that it did not at any time exceed 40° C. This temperature was found by other tests to be quite harmless to the virus even when maintained for many days. The time schedule and findings are indicated in table 2.

A rather small number of exposures of the virus to X-rays of light were made; and for the sake of suggestion are included here in table 3. It is not felt, however, that enough work was done in this connection to justify generalizations.

Considering the very great amount of effort which has been made to relate bacteria and the virus of tobacco mosaic, it is somewhat surprising

that a more careful comparison of the effect of light on the two should not have been made.

The foregoing results show clearly that the mosaic virus is strongly resistant to the influence of the entire solar beam of light, as well as to the portion at the extreme end of vibration frequency. This is, of course, in direct contrast to the action of light on bacteria. Bacteria in general are destroyed in from 1 to 2 hours in sunlight and in from 1 to 5 minutes by ultra-violet rays. Enzymes, on the other hand, are very resistant to

TABLE 3.—*The effect of X-rays on the virus of tobacco mosaic*

Time of exposure in minutes	No. plants inoculated	No. plants infected	Per cent of infection
None	10	10	100
5	10	8	80
10	10	10	100
20	10	10	100
30	10	9	100

the action of light, in some cases appearing not to be completely inactivated by indefinite exposure, although reduced in their zymogenic power. Thus it appears that in this particular the virus behaves like an enzyme and not like a bacterium.

EFFECT OF HEAT ON THE VIRUS OF TOBACCO MOSAIC

Considerable attention has been given to the action of heat on the virus of tobacco mosaic. This relation has been studied from two points of view: first, the temperature required to inactivate or kill the virus; and, second, the influence of heat on the progress of the disease in the host plant.

The determination of the thermal death-point of the virus under any given set of conditions is, of course, relatively easy, and this was ascertained with some degree of certainty in early studies of mosaic. Mayer (20) claimed that heating the virus to 60° C. did not destroy it; at 65°–75° C. it was attenuated, and heating to 80° C. killed the infective agent. Beijerinck (5) states that heating to the boiling point destroyed the disease-producing power of the virus. Chapman (7) found that heating at 80° C. for a short time killed the virus. Raciborski (23) showed that heating the sap from a diseased plant for 5 minutes at 62° C. did not cause it to lose its infective power. When heated to 100° C. for 15 minutes, however, the infective quality was lost.

As will be noted, three of these reports give no time limit to the heat application. In Raciborski's findings the time control is definite; yet the interval between 62° C. for 5 minutes and 100° C. for 15 minutes is very

wide and leaves a considerable range of possibilities. The second aspect of heat relation of the virus, namely, the effect of temperature on the progress of the disorder in the host, has been carefully studied by Johnson (16). He found that the symptoms of the disease in the plant could be completely masked by subjecting it to a rather radical change of temperature.

TABLE 4.—*The effect of long-time exposure of the virus of tobacco mosaic to different temperatures*

Temperature °C.	Time of exposure in days	No. plants inoculated	No. plants infected	Per cent of infection
15	10	5	4	80
25	10	5	5	100
35	10	5	4	80
45	10	5	5	100
15	16	5	5	100
25	16	5	5	100
35	16	5	4	80
45	16	5	5	100
55	16	10	8	80
65	12	10	9	90
75	4	10	8	80
75	8	10	8	80
80	1	5	5	100
80	2	5	5	100
80	3	5	3	60

These symptoms would reappear, however, if the plant was returned to the original heat environment. This phenomenon will be discussed later.

Experiments were conducted to determine the effect of long exposure of the mosaic virus to temperatures ranging from 15° C. to 84° C., the part that moisture plays in the destructive action of heat, and the thermal death-point of the virus when heat is applied for 10 minutes. In the tests made to determine the influence of long subjection of the virus to heat, 15° C. was selected as a starting point and 10 days as the time limit. With the time factor constant, the temperature was shifted upward until 45° C. had been reached. After preliminary results had been secured, both the temperature and time factors were varied according to the dictates of judgment as to what outcome might be looked for. In these experiments the virus was contained in freshly expressed juice of diseased plants. In all cases the infectiousness of the juice was ascertained at the beginning of the tests. Temperature control was secured in some of the experiments by the use of an electrical incubator, in others in an electrically heated and stirred water

TABLE 5.—*The comparative effect of heat on the virus of tobacco mosaic in solution and dry state*

Condition of virus	Temperature °C.	Time of exposure in hours	No. plants inoculated	No. plants infected	Per cent of infection
Solution	82	2	10	10	100
do	82	4	10	10	100
do	82	6	10	10	100
Dry	82	2	10	10	100
do	82	4	10	10	100
do	82	6	10	10	100
Solution	82	10	10	9	90
do	82	12	10	4	40
do	82	24	10	5	50
Dry	82	10	10	9	90
do	82	12	10	4	40
do	82	24	10	1	10
Solution	83	24	10	0	0
Dry	83	24	10	0	0
Solution	84	24	10	0	0
Dry	84	24	10	0	0

bath. In both methods the control was very satisfactory. Temperatures were always taken inside the test tube containing the virus.

In the experiments conducted to determine the relation of moisture to the destructive action of heat, equal amounts of powdered, diseased leaves were placed in test tubes. Half of the tubes were supplied with measured amounts of water while the other half were left dry during the period of heat application. The tubes were subjected to the heat in pairs, and after subjection the dry tubes were given the same amount of water as the others contained. In this way the dilution factor was made constant.

TABLE 6.—*The thermal death-point of virus of tobacco mosaic on 10-minute exposure basis*

Temperature °C.	Time of exposure in minutes	No. plants inoculated	No. plants infected	Per cent of infection
83	10	10	8	80
85	10	10	5	50
87	10	10	3	30
88	10	10	3	30
89	10	10	1	10
90	10	10	0	0
95	10	10	0	0

The 10-minute thermal death-point was determined on freshly expressed plant juice contained in test tubes, the only variable being the temperature.

Representative results of these experiments are given in tables 4, 5, and 6.

It is evident that the mosaic virus is very resistant to heat as compared with non-spore-bearing bacteria or bacterial toxins, withstanding temperatures of from 65° C. to 70° C. for many days without suffering definite reduction in its infective power. Even 80° C. for 3 days reduced it but 40 per cent in its disease-producing quality. Although there is considerable variation among enzymes in respect to their heat resistance, in general a higher temperature is required to inactivate them than is necessary to kill vegetative cells of bacteria. In this respect, then, the virus behaves less like bacteria than like enzymes.

The presence of moisture at the time of heat application appears to have no influence on the temperature required to inactivate the virus. This response is quite unlike that of bacteria, which uniformly yield more readily to heat in the presence of moisture. This latter reaction is held to be due to the fact that the coagulation point is lowered by moisture. If the virus is of bacterial nature it would seem that it should respond to the coagulation principle as other bacteria do.

The suppression of morbid symptoms in the host plant by a change of temperature may have at least two hypothetical explanations. In the first place the temperature may be sufficient to prevent growth and multiplication of the virus in the plant and thus check its harmful influence. Or the heat may influence the metabolism of the host plant in such a way as to overcome temporarily the action of the virus. It appears not impossible that the virus is a product of the plant's own growth. The interference with the growth of the plant might prevent its production of the harmful agent or virus. The progress of the disease is associated with the growing portions of the plant, and, if growth is seriously interfered with, the disease would in turn be checked. It is certain that a temperature sufficient to mask the disease symptoms is not high enough to destroy the virus when separated from the host.

EFFECT OF ANIMAL INOCULATION ON THE VIRUS OF TOBACCO MOSAIC

The literature appears to contain no references to the use of animal inoculation in the study of the mosaic virus. It is, of course, well known that non-pathogenic bacteria soon disappear from the blood of animals which have received them into their circulation. It is important to know what fate would overtake the virus if introduced into an animal's circulation.

The interrelation between plant and animal pathology perhaps has not been so strongly emphasized as could be desired; and the methods employed in one field have not been used to the limit of their usefulness in the other. Virus diseases seem to offer mutual ground for investigation as far as their causative agents are concerned. Although positive knowledge is still lacking as to whether viruses should all be placed in the same category, they are usually classed together. Such facts as have been secured indicate, however, that generalizations in this direction are largely unwarranted without fuller information.

Experiments were conducted to determine whether the mosaic virus could be recovered from the blood-stream of rabbits into which it had been injected. Chamberlain candle filtrates were used as inocula into the ear vein of rabbits which weighed 1500 to 1700 grams. Beginning with a dose of 1 cc., gradual increases were made until 3 cc. doses were given. At the end of a specified time after injection, samples of blood were drawn from the vein of the ear opposite to that which had received the injection. These samples were tested for the presence of infective virus. Results of these tests are presented in table 7. Recovery of the virus was not possible at any time at which samples were taken from the animals' blood.

TABLE 7.—*The effect on the virus of tobacco mosaic of injecting it into the blood-stream of rabbits*

Injection filtrate cc.	Time in minutes in the animal	No. plants inoculated	No. plants infected
1	20	10	0
1	30	10	0
2	20	10	0
2	30	10	0
2½	20	10	0
2½	30	10	0
3	20	10	0
3	30	10	0

In seeking an explanation of this disappearance of the virus from the circulation of the rabbit, tests were made to determine whether the destructive action was due to the blood. Blood was drawn from the ear vein of normal animals and immediately mixed with equal amounts of filtrates from infected plants. Plant inoculations were made from this mixture according to the schedule given in table 8. In other tests the blood was mixed with expressed infectious plant juice which had not been filtered (table 9).

It is shown that the fresh blood of a rabbit is capable of destroying the infective quality of the mosaic virus. According to the results of Freiburg

(10), when talc is mixed with plant solutions containing the virus, the latter is destroyed. This is held to be a case of selective adsorption. Duggar[2] has shown that the juice of pokeweed will act on the mosaic virus in a way similar to that of the action of talc. It may well be that, in the case of the destructive action of animal blood, we have encountered another example of selective adsorption.

Whether it is the serum or the cellular elements of the blood which inactivates the mosaic virus cannot be stated at present. Results secured by a few inoculations with centrifugally separated serum and cells mixed with the virus were inconclusive.

TABLE 8.—*The effect of drawn rabbit blood on the virus of tobacco mosaic in filtrate*

Blood and filtrate	Time in hours in contact	No. plants inoculated	No. plants infected
Equal parts	½	10	10
Equal parts	6	10	7
Equal parts	12	10	0
Equal parts	24	10	0
Filtrate alone	.....	10	8

TABLE 9.—*The effect of drawn rabbit blood on the virus of tobacco mosaic in raw juice*

Blood and raw juice	Time in hours in contact	No. plants inoculated	No. plants infected
Equal parts	2½	10	10
Equal parts	24	10	0
4 to 1	2	10	7
4 to 1	24	10	5
Juice alone	.....	10	10

DIALYZABILITY OF THE VIRUS OF TOBACCO MOSAIC

Since the original studies of Iwanowski (15) showed that the causal agent of tobacco mosaic would pass through fine earthen filter candles, numerous filtration processes have been applied to its study. The passage through such filters has been regarded as merely mechanical, depending on the relative size of the bodies to be filtered and the porosity of the candle, and has not been looked upon as a means of separating different kinds of suspensoids from each other on the basis of their particular colloidal properties. For the latter purpose resort has usually been made to various kinds of dialyzing membranes such as parchment paper, collodion sacs, and

[2] Unpublished results.

animal membranes. In connection with the study of the mosaic virus only two references to the use of such membranes are to be found in published form. Hunger (14) makes the mere statement that the virus will pass through parchment. Duggar and Karrer (8) introduced collodion sacs into a series of studies on the size of the particles of the mosaic virus. These proved unsuccessful, however, and were given a single trial and discarded. It would seem possible at least to differentiate between the simple colloids and any organized cellular body. On the basis of the colloidal chemists' conception, proteins, as well as other colloids, consist of micellae or aggregates of molecules. The larger of such aggregates must be broken up in advance of passage through dialyzing membranes. Formed proteins

TABLE 10.—*The dialyzability of the virus of tobacco mosaic through different grades of collodion sacs*

Grade of sac: percentage of alcohol	No. plants inoculated	No. plants infected	Per cent of infection
20	10	9	90
30	15	8	53+
40	15	7	46+
45	10	0	0
50	15	0	0
60	10	0	0
70	10	0	0
Non-dialyzed juice	10	9	90

are thought to consist of relatively large groups of molecules, and are practically undialyzable. Our present notion of a living entity being that of complex protoplasm carrying a very considerable amount of proteins would lead to the conclusion that such entities could not possibly go through a membrane which would not allow their components to pass. It would require that such bodies be disrupted, and their protein constituents themselves would have to be broken into minor groups of molecules before even they could dialyze. Now we can not think of a cell sustaining such disintegration and retaining its integrity as a cell. Conversely, if a body should be found to have passed such dialyzing membranes as mentioned and still possess its original properties, it could not be regarded as a living protoplasmic unit.

According to the findings of Eggerth (9) and others, collodion sacs of different dialyzing properties may be made by varying the ratio of alcohol to ether as a solvent for the collodion. The higher the relative percentage of alcohol in the mixture, the easier will be the dialytic transfer of sub-

TABLE 11.—*Collected results of dialysis experiments on tobacco mosaic virus with different grades of collodion sacs*

Grade of sac: percentage of alcohol	No. plants inoculated	No. plants infected	Per cent of infection
20	30	20	66.6
25	20	15	75
30	50	8	16
35	40	9	22.5
40	40	10	25
45	70	3	42
50	110	5 (4 from 1 sac)	4.5
50 acid	80	25	31
50 salt	20	0	0
50 NaOH	20	0	0
55 acid	40	14	23 +
55	10	0	0

stances. Thus, a sac made from collodion dissolved in 75 per cent alcohol and 25 per cent ether solution will permit passage of a substance which would be entirely blocked by a sac made from 25 per cent alcohol and 75 per cent ether solution.

The interest we had in finding a sac which would permit the passage of the virus of mosaic led to the making of a graded series from 20 to 70 per cent alcohol solutions, ascending by intervals of 5 per cent.

The manipulations followed in making the collodion sacs were essentially those described by Gates (11) in a recent publication. Eight per cent of collodion is dissolved in the desired mixture of completely dehydrated alcohol and ether. The sacs are moulded in short test tubes, and a

TABLE 12.—*The influence of chemicals on the dialyzability of virus of tobacco mosaic through different grades of collodion sacs*

Grade of sac: percentage alcohol	Chemicals used	No. plants inoculated	No. plants infected	Per cent of infection
50	HCl, 2 per cent, N/20	30	7	23 +
50	NaCl, 1 per cent	20	0	0
50	No chemicals	10	0	0
55	HCl, 1 per cent, N/20	40	14	35
55	NaOH, 1 per cent, N/20	30	0	0
55	Glucose, 1 per cent	20	1	5
55	No chemicals	20	0	0
Non-dialyzed juice		20	20	100

glass tube is inserted inside the sac within the test tube. The sac is then removed with the glass tube to which it adheres loosely. The tube is withdrawn from the bottom of the sac about one inch. After the sac in contact with the tube dries somewhat it will adhere tightly, and the lower portion will hang suspended below the glass tube.

In use, the glass tube with its sac is supported above a vessel containing the expressed juice from a plant known to be affected with mosaic. The sac is then partly filled with distilled water and lowered into the juice so that the free end is submerged. In this position it remains a specified length of time, when the contents of the sac are taken out with a pipette. The solution thus secured, consisting of the distilled water and whatever substance may have passed through the membrane, is used to inoculate young tobacco plants.

TABLE 13.—*The influence of electrical current on the dialyzability of virus of tobacco mosaic through collodion sacs*

Grade of sac: percentage alcohol	Direction of current flow	No. plants inoculated	No. plants infected
50	From juice to distilled H_2O	20	0
50	From distilled H_2O to juice	20	0

Tables 10–13 are representative of what was found in a very long series of experiments. It is observed that the mosaic virus will pass with a fair degree of certainty through sacs made from a solution containing from 20 to 45 per cent of alcohol as a solvent for the collodion. Sacs having a higher percentage of alcohol than 45 almost never allowed the virus to pass unless the juice carrying it had its pH value changed to the acid side. In this latter respect the virus behaves as a typical colloid, as it is known that a change of reaction slightly to the acid or alkaline side of the isoelectrical point will encourage colloids to pass such membranes. The addition of alkaline solutions to the juice containing the virus did not induce a passage of the latter through sacs impervious to it in the untreated juice. This is no doubt due to the fact that the juice is normally on the alkaline side of the isoelectrical point.

The addition of salt and sugar to the solution containing the virus did not change the dialyzing property of the virus. The results were likewise negative when an electrical current was passed across the membrane which separated the plant solution and virus from distilled water. The current was passed in both directions, that is, from the juice to the water and from the water to the juice. Hydrophylic organic colloids, according to Bechhold (4, p. 77), do not pass in any definite direction in an electrical field.

They acquire a definite polarity relation only on the addition to the solution of an electrolyte. If the virus in the plant juice acts as a colloid, we should not expect the passage of the current to change the property of movement through the sac if no electrolyte had been supplied. The fact, observed here, that the current does not increase the movement of the virus through the membrane points to a similarity between it and colloids.

EFFECT OF BACTERIA ON THE VIRUS OF TOBACCO MOSAIC

The fate of the mosaic virus in the soil is of very considerable economic importance and has received much attention. The reported results are not

TABLE 14.—*The influence of bacterial growth on the tobacco mosaic virus when cultured in equal parts of bouillon and filtrate*

Organism	Cultivation temp. °C.	No. plants inoculated	No. plants infected	Per cent of infection
B. subtilis	37	5	2	40
B. coli	37	5	1	20
B. suipestifer	37	10	2	20
B. prodigiosus	37	10	1	10
B. hartlibii	37	10	5	50
B. proteus vulgaris	37	10	1	10
B. anthracoides	37	10	3	30
B. ruber balticus	20	20	0	0
Sarcinia lutea	20	20	3	15
B. aerogenes	20	20	2	10
Cellulose fermenter	65	40	1	25
No bacteria	20	35	21	60
Check plants 30		0	0	0

concordant, yet on the whole they give one the impression that the virus is soon lost when introduced into field soils, or moist soils. Beijerinck (5) claimed that it was not destroyed by remaining in the soil over winter. Koning (18) could not produce the disease in healthy plants by inoculating them with soil solutions from fields where diseased plants had grown. Allard (1) secured results which indicated that the virus is soon lost or destroyed in field soil. In solutions of plant juice the virus is known to withstand the process of decomposition of the juice and remain infectious.

In the soil, of course, the virus is subjected to the action of a very complex biological condition, bacterial, enzymatic, and protozoan. It is manifestly impossible to know anything concerning the relative influence of any one of these factors under such conditions. Experiments were undertaken to show the individual effect of certain bacterial species on the virus. In all of the experiments conducted in this connection equal parts of filtrates

TABLE 15.—*The destructive action of selected bacteria on the virus of tobacco mosaic*

Organism	No. of inoculations	No. of infections	Per cent of infection
Sarcinia lutea	40	17	42.0 +
B. ruber balticus	40	7	17.0 +
B. proteus vulgaris	40	3	7.5
B. aerogenes	40	4	10.0
Cellulose fermenting bacillus	40	1	2.5
Check filtrate and bouillon	40	30	75.0
Filtrate alone	40	31	77.0 +

of diseased plant juice and sterile bouillon were placed aseptically in test tubes. After the mixture was shown to be free from bacteria it was seeded with the organism to be tested. Sufficient time was allowed for the bacteria introduced to complete their growth in the medium. Plants were then inoculated with the culture. The results are shown in table 14.

These results show that all of the bacteria included in the foregoing experiments possess some power to inactivate the virus of mosaic. Some show very little capacity in this direction, as in the case of *Bacillus hartlibii,*

TABLE 16.—*The effect of heat on enzymes, bacteria, animal viruses, and the mosaic virus of tobacco in moist and dry conditions*

Enzymes, bacteria, toxins, and viruses	Destruction temp. in degrees C.		Authority
	Wet	Dry	
Sucrase	55–75	160	Kayser (17, p. 60)
Amylase	75–80	130	do
Trypsine	70	100	do
Lipase	60	.....	do
B. tuberculosis	60	100	Hiss and Zinsser (13)
S. cholera	52	.....	do
B. typhosus	60	.....	do
M. gonorrhea	62	.....	do
B. anthracis spores	100	140	do
Diphtheria toxin	60	.....	Oppenheimer (22)
Botulinus toxin	58	.....	do
Symptomatic anthrax toxin	50	.....	do
Horse anaema virus	58	.....	Buchanan (6)
Fowl plague virus	55–60	.....	do
Cattle plague virus	58–60	.....	do
Hog cholera virus	60–70	.....	do
Tobacco mosaic virus	88–90	88–90	do

TABLE 17.—*The effect of light on enzymes, bacteria, toxins, and the mosaic virus of tobacco*

Enzymes, bacteria, toxins, and viruses	Loss of activity in per cent	Time of exposure	Authority
Malt diastase	68	.....	Hill (12, p. 97)
Saliva diastase	45	.....	do
Plant diastase	8	.....	do
B. tuberculosis	100	1 hr.	Hiss and Zinsser (13)
M. gonorrhea	100	5–10 min.	do
Diphtheria toxin	100	1 hr.	do
Anthrax toxin	0–5	indefinite	Oppenheimer (22)
Botulinus toxin	100	1 hr.	do
B. anthracis spores	100	1 hr.	Arloing (3)
Tobacco mosaic virus	90	36 hrs.	

while others completely destroy the infectiousness of the virus, as, for instance, *B. ruber balticus*. The organisms used were selected without reference to their biological qualities, and there seems to be no possibility of distinguishing the vigorously destructive group from the non-destructive group on the basis of any recorded physiological or morphological characteristics.

A more extensive study was given to four selected members of the group of bacteria which had shown greatest capacity for destroying the virus. The figures given in table 15 for 40 inoculations were compiled from quadruplicate cultures of each bacterium, ten plants being inoculated from each culture. Duplication was very good in all cultures with a given organism.

The results merely emphasize the fact, already alluded to, that certain bacteria in pure cultures have a decided ability to overcome the disease producing faculty of the virus of tobacco mosaic.

TABLE 18.—*The relation of moisture to the coagulation of albumin and destruction of mosaic virus of tobacco by heat*

Material	Percentage water	Coagulation temperature in degrees C.	Death-point in degrees C.	Authority
Egg albumin	100	65	.....	Lewith (19)
do	25	74–80	.....	do
do	18	80–90	.....	do
do	6	145	.....	do
Mosaic virus	100	.....	88–90	
do	Air dry	.....	88–90	

An analysis of tables 16–18 shows that there is considerable variation among the members of all the groups of bodies considered as to their response to heat and light. In regard to the influence of heat, the mosaic virus behaves more as an enzyme than as any of the other classes of bodies. The animal viruses are destroyed uniformly at a comparatively low temperature, while bacteria and toxins fall into an intermediate position between them and the ferments. The mosaic virus displays one characteristic not noted in any of the other substances, namely its indifference to the moisture content as affected by heat. As is seen, the coagulation temperature of albumin depends on its content of water; whereas the mosaic virus is destroyed by heat independently of moisture. This indicates that its inactivation is not due to coagulation. Both mosaic virus and enzymes are very resistant to the action of light. Bacteria on the other hand are relatively sensitive to light.

One is led to conclude from these considerations that the mosaic virus of tobacco is more nearly related to enzymes than to either bacteria, animal viruses, or toxins as far as this kind of evidence permits generalization.

THEORETICAL DISCUSSION

Granting the accuracy of observation and validity of conclusions set out above concerning the viability of the causal agent of tobacco mosaic, we are obliged to seek a non-vitalistic explanation of the diseased condition of the plants. In so doing at least two hypothetical possibilities present themselves. It is generally conceded that in some way the virus is multiplied. How then may such increase be accomplished if the active agent is not a living entity? First, it may be conceived as a product of the plant's own metabolic activity under the influence of some disturbing factor introduced into the plant body, namely the virus produced by other plants similarly affected. Thus we invoke the principle of auto-catalysis. Autocatalyses of a purely chemical nature are well known, and organic autocatalyses are also recorded. The enzymatic transformation which takes place in the endosperm of germinating grain, as in the wheat grain, is perhaps of this character. The parallel multiplication ratio of the bacteriophage and the bacteria which it lyses during the lytic phenomenon, supported by other evidence, is being regarded as an auto-catalysis by an increasing number of investigators. Certain manifestations during the progress of the mosaic disease lend themselves readily to such an explanation. Among these may be mentioned the incubation period, the apparent modification of the virus during its sojourn in different hosts, the correlation between the progress of the disease, and cell division in the meristem tissue and the fact that the plant does not recover and is not killed by the disorder.

A second hypothesis may be stated as follows. It would seem to be essential to the theory of evolution to assume that somewhere, somehow, living entities sprang from inert matter. We distinguish between living and non-living things by noting certain qualities in one that are not observable in the other. In general these qualities are reproduction, nutrition, adjustment, and response to stimuli. These properties appear to be distinct qualities, that is, not dependent upon each other. These separate factors of life must have been acquired somewhere along the path of emergence from inert to living conditions. There is nothing in the logic of the case to prevent the assumption that these qualities may have been acquired one by one rather than simultaneously. There is nothing, then, to preclude our thinking that there may still be bodies, or aggregates of molecules, which are possessed of any one or more of these factors, situated along the way of evolution, bodies so to speak between the living and the dead as life is now conceived. Such bodies might well be able to reproduce without capacity for adjustment, response, or possibly nutrition.

The mosaic virus might be possessed of two of these qualities, namely reproduction and nutrition, or even that of reproduction alone, increase in quantity being some form of additive process incited by the host plant. The plant might thus add molecules to the initial foreign group up to the point of reproduction, the one function of life inherent in the initial foreign group of molecules. Now it is possible to see how this continuous addition of certain molecules to the foreign body could disturb the normal metabolism of the host plant. Molecules thus being taken out of their normal course would leave the plant without its due quota and thus prevent the maintenance of the nutritive balance normal to the growing plant. Or if, in addition to the quality of reproduction, the virus group of molecules should have that of nutrition as well, it might be seizing upon particular plant molecules appropriating them to itself. In either case the effect upon the host plant would be the same. Such a view, while entirely hypothetical, seems not to be outside the realm of possibility.

Mrowka (21) and Andriewsky (2), working on chicken pest independently and with different methods, came to the conclusion that the virus is a globulin. The same conclusion was reached by Sangiorgi (24). It is not impossible that the mosaic virus of tobacco may be of similar nature, that is, a protein of very simple kind, having the characteristics of an enzyme.

SUMMARY

1. Experiments have been conducted to show the influence of light on the virus of tobacco mosaic. The results show that it is very resistant to the action of light. Exposure of the virus to direct sunlight for 36

hours destroyed 90 per cent of its infectiousness. Ultra-violet light inactivated the virus in one hour. X-rays do not appear to injure it.

2. Exposure of the virus of tobacco mosaic to 80° C. for as long as 20 days did not completely destroy it. The thermal death-point of the virus on the 10-minute exposure basis is between 88° and 90° C. No relation between the moisture content of the material carrying the virus and the killing temperature could be detected.

3. It was not possible to recover the virus of tobacco mosaic from the bloodstream of a rabbit, into which it had been injected, at any time between 3 and 30 minutes. The injection caused no detectable reaction on the part of the animal. Drawn blood of the rabbit, when mixed with plant juice containing the virus, inactivated the latter. Results with cow and horse blood are less definite.

4. The virus of tobacco mosaic has been found to pass through certain grades of collodion sacs and not through others. A slight change of reaction of the juice containing the virus, to the acid side of the isoelectrical point, induced the passage of the virus through sacs impervious to normal juice. The passage of an electrical current across the sac does not cause the virus to dialyze. Sugar, salt, and alkalies are equally ineffective in this direction. These results lead to the conclusion that the virus of tobacco mosaic cannot be as complex a body as a biological cell, therefore not a living entity in the present accepted sense.

5. Certain bacteria have been found to be very destructive to the virus of tobacco mosaic when cultivated in a solution containing it. Other species are but slightly, if at all, harmful to it. Apparently there is no biological characteristic by means of which the two groups of bacteria can be separated. Therefore no suggestion can be made as to how the inactivating effect on the virus is produced.

6. The evidence gained by these experiments seems to point to the virus of tobacco mosaic as a non-living thing, possibly a very simple colloid, possibly also of protein nature and having enzymatic characteristics.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN.

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ATTEMPTS TO CULTIVATE THE TOBACCO MOSAIC VIRUS

M. C. GOLDSWORTHY

Olitsky[1] has obtained evidence that the virus which causes the mosaic disease of tobacco and tomato can be cultivated *in vitro*. The virus, when transferred in dilutions to a suitable culture medium, which consists of the clear filtrate of normal tomato tissue, increases in potency after an incubation period at 30° C. The results of his experiments seem to justify the conclusions made. Apparently there was an increase in the causative agent.

Mulvania[2] carefully repeated the work. Tobacco plants inoculated with incubated tomato mosaic virus failed to show an increase in infection over that caused by the unincubated material. Furthermore he showed that diluted virus suspended in distilled water and incubated gave as much infection as the incubated material in the culture medium. Mulvania concludes that the mosaic virus of tobacco and tomato can not be cultured according to the method outlined by Olitsky.

The findings of these two workers suggest the need of further work on the subject. The writer has repeated the work, using tobacco plants throughout the experiment. Olitsky used tomato plants; Mulvania used tomato and tobacco. Since the disease of both plants is caused by the same virus, either system or a combination of both may be used.

The experiment was carried out under greenhouse conditions. The tobacco plants were grown from seeds and potted in 6-inch pots. Ten plants were used for each test.

The experiment was carried out in two series. The virus for the first series was obtained from dried diseased tobacco leaves sent by Prof. A. V. Osmun of the Massachusetts Agricultural College 18 months previously. The virus for the second series was secured from diseased tobacco plants resulting from the first inoculations.

In both cases the virus was secured by mincing and crushing the mosaic tissues with distilled water in a mortar. The resulting fluid was then passed twice through Berkefeld filters of V porosity. The clear filtrate was tubed and incubated for 10 days at room temperature in order to test its sterility.

The culture medium of Olitsky's was obtained by mincing, crushing and filtering normal tobacco tissues as above. The clear solution gave,

[1] Olitsky, P. K. Experiments on the cultivation of the active agent in mosaic disease in tobacco and tomato plants. Jour. Exp. Med. 41: 129–136. 1925.

[2] Mulvania, M. Cultivation of the virus of tobacco mosaic by the method of Olitsky. Science 62: 37. 1925.

using the hydrogen electrode, a pH of 5.9. The solution was tubed in 10 c.c. lots and allowed to remain in the incubator for 10 days at 30° C. before inoculating with the dilutions of the mosaic virus. The tubes remained clear after this incubation period.

After this preliminary sterility test, the culture tubes were inoculated with dilutions of the virus solutions. The dilutions were made by transferring 0.1 c.c. of virus solution to 10.0 c.c. of the culture fluid. Dilutions of 1/100, 1/10,000 and 1/1,000,000 were obtained. Undiluted and diluted tubes were then incubated for 8 days at 30° C.

One set of 10 plants in each series was inoculated with the original virus solution after passing through the filters. The incubated material was inoculated into sets of 10 plants. The inoculations were made by injecting the material from the culture tubes into the axils of the leaves with sterile pasteur pipettes. Check lots of plants were placed among the inoculated sets. These were inoculated with sterilized distilled water.

Table 1 gives the number of the series, magnitude of the dilution, and the number of diseased plants.

TABLE 1.—*Results of inoculating tobacco plants with mosaic virus of varying dilutions*

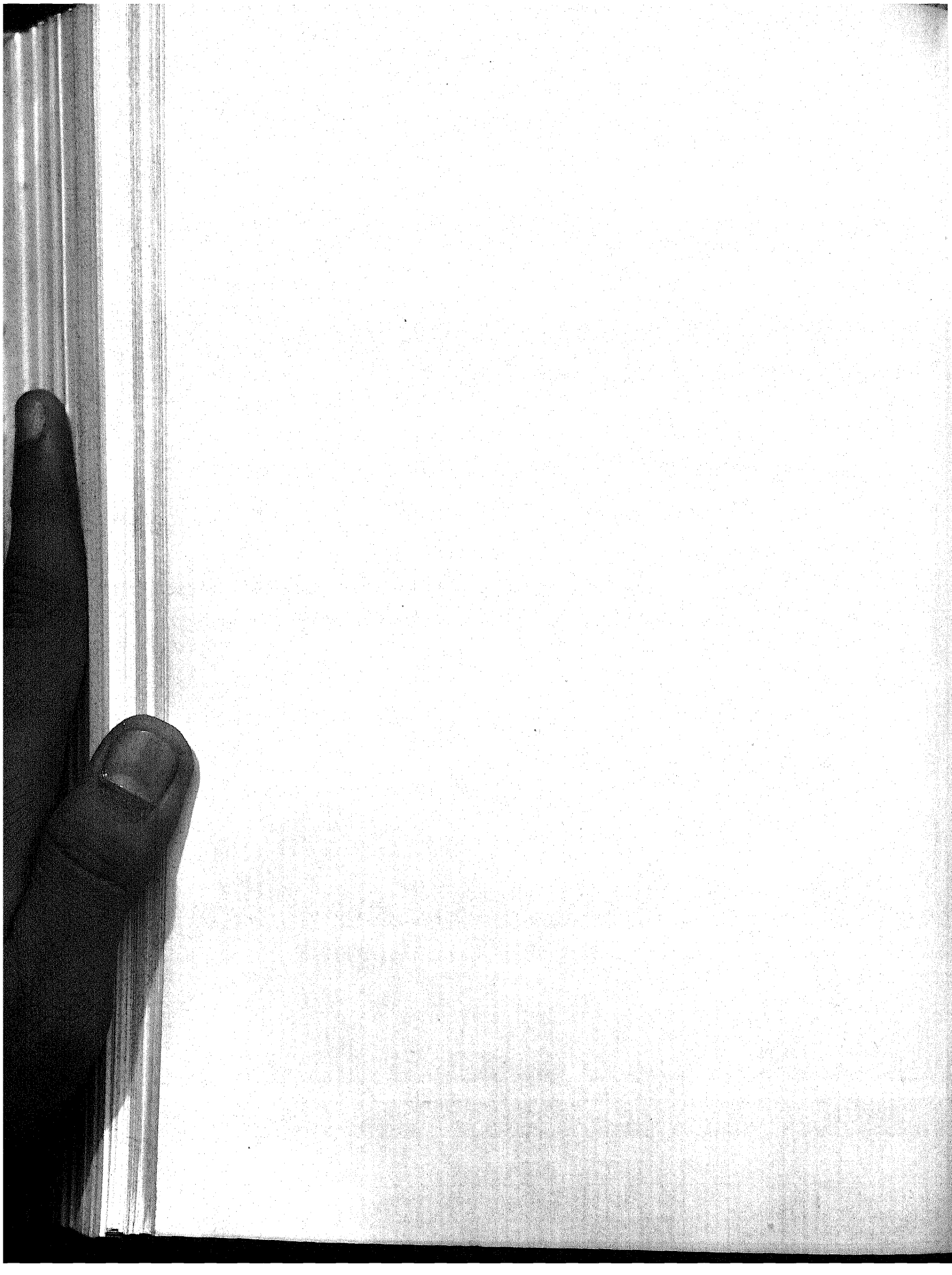
Dilutions	Incubation for 8 days	Series 1 Mosaic virus obtained from dried leaves stored 18 months. Ten plants in each set.	Series 2 Mosaic virus from a diseased plant from Series 1. Ten plants used in each.
		No. diseased plants	No. diseased plants
Undiluted		5	10
do	30° C.	6	10
1/100	do	2	7
1/10,000	do	0	3
1/1,000,000 A	do	0	1
1/1,000,000 B	do	1	0
1/1,000,000 C	do	0	0
1/1,000,000 D	do	0	0
1/100 of A	do	0	.....
1/10,000 of A	do	0	.....
1/1,000,000 of A	do	0	.....
Distilled water (check) A		0	0
Distilled water (check) B		0	0
Distilled water (check) C		0	.....

CONCLUSIONS

Olitsky's findings have not been verified during the course of the experiment on tobacco mosaic. Diluted tobacco mosaic virus did not increase its

potency when incubated in the culture medium described by Olitsky. According to the findings of Olitsky one would expect the diluted cultures to give, after the incubation period, as much infection as the original undiluted virus solution. Especially should this be so with the lower dilutions. The results of this experiment support the findings of Mulvania. There is a gradual decrease in the number of infections as the virus is diluted, and in only two cases out of eight did the 1/1,000,000 dilutions give any infection. No infections occurred when the 1/1,000,000 incubated dilutions were further diluted.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIF.



STUDIES ON THE SPOT DISEASE OF CAULIFLOWER; A USE OF SERUM DIAGNOSIS

M. C. GOLDSWORTHY

This disease, first described by Lucia McCulloch (3) and later by Erwin F. Smith (4), appears to be of growing economic importance throughout the cauliflower plantings of the San Francisco Bay Region in California. In the fields and in the local markets a great many of the heads are affected. This is especially so at the height of the season, during February and March. At this time nearly all of the heads are spotted.

The disease is easily recognized by the small brown or dark brown spots that appear on the inflorescence or edible part of the plant. These spots vary in size, sometimes reaching several millimeters in diameter; they are at first firm but later become soft. As the disease progresses, invasion by saprophytic or secondary organisms occurs, and the decay becomes characterized by a very offensive odor. Sometimes the heads are slightly spotted, but during severe attacks the whole inflorescence becomes covered with these brown, firm areas. The spots are confined to the epidermal and adjacent layers of parenchyma cells, and very seldom are they found affecting the deeper tissues.

The observations of the writer indicate that the disease is commonly one of over-mature cauliflower heads. Occasionally it occurs after injuries. In the field one finds the spots on heads that have been mature for a period before picking. Since size is a market factor of some importance, the tendency is for the grower to try to get as large a head as possible. For this reason many of the heads are allowed to remain in the field longer than necessary, and consequently become spotted.

Sharp frosts, such as California experiences during the months of January, February and March, indirectly cause considerable damage to the cauliflower inflorescence. There seems to be no direct frost damage as is usually evidenced by blackened tissue, but nevertheless the brown spots increase considerably in numbers after these sharp changes in temperature. There seems to be just enough injury to the epidermal cells of the fleshy inflorescence to allow the invasion of the organism causing the brown spots.

The writer has observed repeatedly, during the winters of 1922–23, 1923–24 and 1924–25, that, following these changes in temperature, considerable spotting of the cauliflower heads occurred in the fields. On the market stands the heads were often 100 per cent affected. During these

periods the infections were general, and seldom was one able to find a head of cauliflower free from spots.

The writer has repeatedly isolated the causal organism, *Bacterium maculicolum*, described by McCulloch. This organism can readily be isolated in pure cultures from the spots when they are small and firm. When the spots are soft, other organisms appear on the plates. The isolated organism, when sprayed on cauliflower heads under sterile laboratory conditions, readily reproduces the disease. The writer has repeatedly carried it through three generations on cauliflower heads.

The organism isolated by the writer agrees culturally and morphologically with that described by Miss McCulloch. The writer did not succeed in producing the black spots on young cauliflower seedlings as McCulloch was able to do. She noted that only recently isolated organisms were capable of producing these spots and attributed the non-production of lesions by older cultures to a loss of virulence due to age. The writer used both young and old cultures without success.

The organism is gram negative, stains readily with the ordinary dyes, and is non-acid fast. A short rod, occurring usually in pairs, sometimes as short chains. Motile by two to five polar flagellae (Plimmer and Paine method). Gelatin moderately liquified. Lactose, saccharose, maltose, dextrose, xylose, mannite, and glycerin not fermented. Acid not formed in above. Slight acid formation in glucose. Strictly aerobic (confined to the open arm of fermentation tube). Grows moderately on beef bouillon agar slants, profusely on Czapeks synthetic sugar media. Forms a yellow-green pigment in gelatin, beef bouillon and in solid media. No nitrite or indol formed. Grows readily at 37° C., also at 4° C. or ice box temperature. No spores are formed. Milk is peptonized. Agglutinins are readily incited in the serum of rabbits. A titre of 1/10,000 was obtained.

PERSISTENCE IN SOIL

Since many of the cauliflower heads are infected in the field, it was thought that the organism causing the brown spot might be a soil-inhabiting species. To determine this, an experiment dealing with the occurrence and persistence of this bacterial pathogen in the soil was conducted.

The literature dealing with experiments of this type was found to be rather unsatisfactory. The persistence of pathogenic bacterial organisms in the soil has often been dealt with in a casual way, and many writers have been content to designate the organisms that they were working with as overwintering in the soil without any experimental basis for their conclusions. That it is not easy to study pathogenic bacteria in the soil is obvious to many plant pathologists. The extreme difficulties of isolation of a spe-

cific form from among the various members of the bacterial flora often makes such a study almost impossible.

Fulton (1), working with *Pseudomonas citri,* the citrus scab organism, studied the decline of the organism in the soil. He recognized the difficulties of trying to plate out his organism from the soil, "because of the preponderance of more rapidly growing soil organisms, ordinary plating methods are inadequate for determining the abundance of *Ps. citri* in soil samples and recourse was had to inoculation of punctured mature grape-fruit leaves with . . . washings from the soil." Kotila and Coons (2), working on the method of overwintering in soil of the potato blackleg organism, *Bacillus atrosepticus,* also recognized the difficulties of the isolation of bacterial plant pathogenes from the soil. The difficulty experienced in endeavoring to pick up colonies of the blackleg organism from plates poured from soil samples is of course obvious. ". . . There is such a great number of soil organisms which develop on the plates, very similar to the blackleg organism, that the chances of picking up the right organism are very small." The above workers used the inoculation method for determining the persistence of their organisms in the soil. This method is open to criticism, in that successful inoculations are not always possible. It has been the writer's experience that successful inoculations are rare indeed in comparison with non-successful. The required relation between the parasite and the host may exist only at times and under certain conditions. It seems unsafe, therefore, to base conclusions on the presence of any parasitic organism on chance infections. Something more specific and, at the same time, easy of manipulation should be used in such studies on the persistence of organisms in the soil.

The writer, taking advantage of the high specificity of antibodies, as used by medical bacteriologists, has brought into use the agglutinating antiserum in his present study. High titre antiserums were produced by injecting into rabbits the appropriate antigen, namely, the organism under study. Identification of the isolated organism was made by agglutinating at high dilutions of the specific antiserum. In this manner the identification became easy and at the same time absolute.

The method of producing the agglutinins was as follows: 24-hour-old cultures growing on Bacto-peptone beef bouillon agar slants (pH 6.8) were suspended in 10 c.c. of physiological salt solution (0.85 per cent). The living organisms were injected intravenously into rabbits weighing about 2,500 grams as follows: on the first day 1.0 c.c. of the above saline suspension, 1.0 c.c. on the second day, and 1.5 c.c. on the third. Seven days after the first injection the series of three were then repeated with doses of 2.0 c.c. each day. Sixteen days after the first injection the animals were bled

from the heart. An excellent titre was obtained by this method. Phenolized cultures did not prove potent. The living suspensions proved to be slightly toxic for the rabbits. A little care must be taken during the first period of injections. The above method is one that is used in the medical bacteriological laboratory at the University of California.

The present work consisted of examination of sterilized and unsterilized flasked soil, inoculated with a broth suspension of *B. maculicolum*. The experiment was carried out under laboratory conditions.

METHODS OF EXPERIMENTATION

Erlenmeyer flasks, capacity 250 c.c., in which 150 grams of ordinary campus soil was placed, were used for the experiment. The campus soil is a heavy clay loam in which are dispersed small and large pieces of native rock. This soil had an initial reaction of pH 6.5 as determined by the hydrogen electrode.

Three sets of 24 flasks of soil were used. One set was sterilized for 4 hours under pressure in the autoclave and inoculated with a suspension of *B. maculicolum* growing in beef bouillon broth. Another set was left unsterilized but was also treated with the suspension of organisms. Enough of the suspension was added in both cases to bring the moisture content of the soils to optimum. The last set was sterilized as above but not treated with the suspension. Distilled water was added to these instead of the suspension. This set was used for the checks. All of the flasks were then placed away in a laboratory cupboard for further examination. The flasks were not molested until used for the experiment. As each flask was opened it was discarded.

The isolations were made by placing a few grams of the flasked soil in a tube of sterile beef broth and incubating at room temperature until turbidity developed. Proper dilutions were then made of the culture, using Czapek's synthetic sucrose agar pH 6.6, which proved to be a very suitable substratum for the isolation of this organism, since it grows luxuriantly and gives a greenish pigment in the medium. Characteristic colonies were then selected and isolations made from these on nutrient beef agar slants. The identification was then made by use of the agglutinating anti-serum of rabbits.

Isolations were made each week, for the first four weeks, then every two weeks for sixteen weeks, then every four weeks for the remainder of the year. The experiment was begun September 17, 1924, and was continued until September 17, 1925.

The natural field conditions were not duplicated in the laboratory experiment. The temperature of the laboratory does not change very much

throughout the year. The moisture conditions under which the experiment was conducted obviously show no analogy with those met with in the field. The fluctuating environmental conditions, which no doubt play a great part in the persistence of any organism in the soil, were lacking in this experiment. The competitive influence of the other members of the soil flora may also play a part in this persistence. This competitive factor was encouraged to some extent in the unsterilized flasks. That the competitive factor was not wholly duplicated is recognized by the writer since certain types of the soil flora which might be present under normal conditions were absent.

The moisture content of the soil decreased slowly throughout the experiment. After two months the flasked soil was dry. At the end of the experiment and, in fact, during the last six months, the soil was powdery dry. The writer feels that, under these moisture conditions, the organisms were placed under quite a severe test.

The problem of identification was made simple by the agglutinating antiserum. Since the question of specificity might enter into the criticisms of this paper, the writer tested other organisms that were found growing on

TABLE 1.—*The growth*[a] *of Bacterium maculicolum and other organisms in sterilized and unsterilized soil. Experiment in progress from Nov. 17, 1924, to Nov. 17, 1925*

Time in weeks	Check (sterilized soil)		Inoculated (sterilized soil)		Inoculated (unsterilized soil)	
	B. maculicolum	Other organisms	*B. maculicolum*	Other organisms	*B. maculicolum*	Other organisms
1	–	×	×	–	×	×
2	–	–	×	×	×	×
3	–	×, f	×	–	–	×
4	–	–	×	–	×	×
5	–	–	×	–	×	×, f
7	–	×	×	×	lost	
9	–	–	×	–	×	×
11	–	–	×	–	×	×
15	–	×	×	×	–	×
19	–	–	×	–	×	×, f
23	–	–	×	–	×	×
27	–	–	×	–	×	×, f
35	–	×, f	×	–	×	×
40	–	–	×	–	×	×, f
44	–	–	×	–	×	×
48	–	–	×	–	–	×
52	–	–	×	–	×	×

[a] –, no growth; ×, growth; f, fungous growth.

the plates obtained from the unsterilized series. A few animal pathogenes, *Bacillus coli, Bacillus typhosus* and *Staphylococcus aureus,* as well as a few plant pathogenes, *Bacillus carotovorus* and two types of *Pseudomonas cerasus,* were also tested with the antiserum. No cross agglutination was observed. Furthermore the agglutinating antiserum was used in 1/1000 dilutions, a fact which obviates any error that might creep in from this direction.

Transfers were made from the characteristic colonies, found growing on the plates resulting from each of the inoculated flasks of soil, sterilized and unsterilized, on to nutrient beef agar slants. Transfers were also made from colonies developed on the plates from the check flasks, which proved to be unsterile. Many of the transfers represented colonies that resembled those of the organism inoculated into the soil. As stated before, these transfers were used to check the group relations if such existed. None of these were agglutinated by the specific antiserum in any dilution.

RESULTS

Table 1 shows the occurrence of *B. maculicolum* in the laboratory soil.

The data given in the table show how readily *B. maculicolum* can persist in soil under the conditions of the experiment. This bacterium appears to be well able to compete with the other members of the soil flora. In no case in this experiment was the organism found normally in non-inoculated soil. This was shown in the flasks which were sterilized but which were found later to harbor a flora. Apparently four hours' sterilization at 115° C. under pressure is not sufficient for flasked soils.

NATURAL OCCURRENCE OF BACTERIUM MACULICOLUM IN SOIL

The writer had occasion during the course of another experiment to accumulate cultures from various soils throughout the northern part of California. These soils represented areas devoted to orchards of deciduous fruit trees such as plums, peaches, apricots and pears. In no way were they connected with the growing of cruciferous crops. All of the samples were taken in the field and immediately placed in sterile beef bouillon broth tubes. These were incubated and plated. Transfers were made of the resulting colonies.

One hundred and eighty-seven soil samples were secured and cultured. *B. maculicolum* was isolated from 28 of these.

NATURAL OCCURRENCE ON INSECTS

The red-bordered stink or plant bug, *Euryopthalmus convivus,* is quite common in the above deciduous orchards, but it appears to do no particular

harm. It inhabits these orchards the year round. This insect is particularly active and is found crawling about in the trees and on the soil and in the soil cover. The writer was curious to find what relation this insect had to the dissemination of bacteria which cause the gummosis of stone fruit trees. Aphids are also found commonly on these trees, and these were also examined. Some 65 insects were cultured by placing them in beef broth. Some of the insects were treated with alcohol before placing them in the culture fluid; some were treated with alcohol and then crushed in the culture fluid. No organisms were isolated from these last two sources. Sixty-five isolations were made from the remainder and, of these, ten proved to be *B. maculicolum*, showing that the insect carries the organism on its extremities. No organisms were isolated from the aphids.

SUMMARY

1. The economic importance of the spot disease of cauliflower appears to be increasing in the San Francisco Bay region of California.

2. Sharp frosts, especially during the latter part of the growing season, cause an injury which allows a greater amount of infection.

3. The practice of allowing the heads to become over-mature appears to favor the infection.

4. The cultural characteristics and biological activities of the bacterial organism isolated by the writer agree with those described by McCulloch.

5. Young cauliflower plants failed to become infected with *B. maculicolum* when sprayed with recently isolated strains.

6. Cauliflower heads were readily infected when the organism was sprayed upon the fleshy part.

7. *B. maculicolum* readily incites the formation of agglutinins in the serum of rabbits when injected intravenously. A titre of 1/10,000 was secured.

8. The bacterium was able to persist in flasked dry soil for at least a year.

9. The bacterium was found naturally in soils which were not cropped to cruciferous plants.

10. The red bordered stink bug, *Euryopthalmus convivus*, carries the organism on its extremities and thereby acts as a disseminating agent.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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COLD CHLOROSIS OF SUGAR CANE[1]

JAMES A. FARIS

During the winter of 1924–1925 the writer observed peculiar white bands upon the leaves of Yellow Caledonia cane on the south coast of Habana Province, Cuba. Several fields were thus affected, and every plant seemed to be uniformly marked. These markings were very definite bands across the leaves, in which the chlorophyll had failed to develop. No such markings were noticed in the Cristalina cane growing alongside these fields. The country people attributed these white bands, or "manchas blancas," as they call them, to freezing during a period of cold weather, but this explanation seemed out of the question, as no freezing temperatures have ever been recorded from this locality. These fields were again observed in May, 1925, two months later, and no new markings had appeared, but the areas on the older leaves still persisted. No further evidence of such chlorosis was seen until December, 1925, when bands appeared simultaneously upon several cane varieties in the collections of the Cuba Sugar Club Experiment Station of the Tropical Plant Research Foundation, at Central Baraguá, Cuba. In January of the present year specimens affected with this form of chlorosis were sent to the station from Central Carmita by Mr. J. T. Crawley. Other cases have been observed by the writer in Santa Clara, Habana, and Oriente provinces during the months of January, February, and March. In some cases these have been confused with mosaic by the field men. A study of the contributing causes was then made and experiments were begun in which these white bands were produced under controlled conditions, as hereinafter recorded.

DESCRIPTION OF THE CHLOROTIC BANDS

The bands observed range from 2 to 4 inches in length and vary in width from a narrow strip along the margin of the leaf to bands which completely cross the leaf and have every appearance of being formed in the cornucopia of the leaves. All of the central leaves are thus marked, but in later growth the affected areas separate and hence are quite differently located upon the mature leaves. As will be seen from figures 1 and 2, the whitened bands are relatively lower upon the older leaves and are progressively higher upon the younger ones. The youngest leaf affected usually has the band at, or very near, the tip.

While the bands appear to be very regular in their limits, it will be seen upon close inspection that the borders of the white and green tissues are very

[1] Scientific Contributions No. 3, Tropical Plant Research Foundation.

irregular (Fig. 3). A part of this irregularity is due to cell divisions subsequent to the original exposure. The tissues involved in this band seem to have lost the power to form chlorophyll. The cases where distinct green stripes develop along the veins are interpreted as being due to the normal development of chlorophyll in the deeper lying tissues which escaped the cold injury, especially the chlorophyll-bearing bundle sheath which forms a continuation of the sclerenchymatous tissues around the fibrous strands of the leaf sheath and cane stalk. These thick-walled cells continue to develop chlorophyll even after this power is lost by the surrounding parenchyma.

FIG. 1.—Plant of H-109 cane with chlorotic bands due to cold injury.

Since every indication pointed to the formation of these chlorotic bands while the young tissues of the various leaves were in the cornucopia, experiments were devised to test the theory by keeping melting ice above this point and permitting the "boot" of the plant to stand full of cold water. A funnel of tough paper was tied about the top leaves of 27 different stalks of H-109 and D-1135 varieties of cane and was kept full of ice during a period of three consecutive nights. The ice was applied during the nights of Janu-

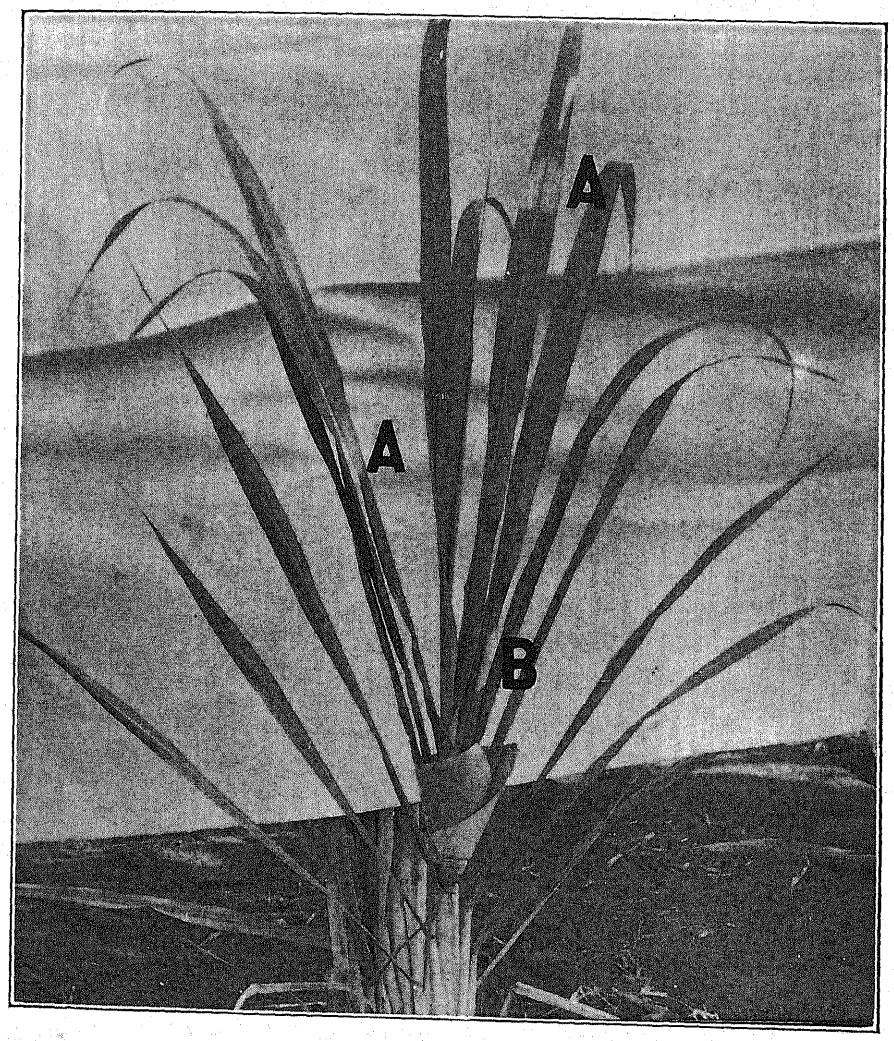

FIG. 2.—Plant of D–1135 cane with (A) chlorotic bands induced by melting ice in the "boot," and (B) induced by cold weather.

ary 22, 23, and 24, 1926. White bands began to develop January 30 upon the treated plants, but no signs of chlorosis developed upon the untreated stalks of the same plants. The bands upon the treated plants were not so marked as bands which developed within a few days after periods of cold weather occurring in December, 1925, and in January and February, 1926. However, artificially produced bands were well marked and definite, as can be seen from the bands indicated as A in figure 2.

A study of the temperatures and rainfall during the season of 1925–26 shows that an outbreak of this leaf banding has occurred in every case where

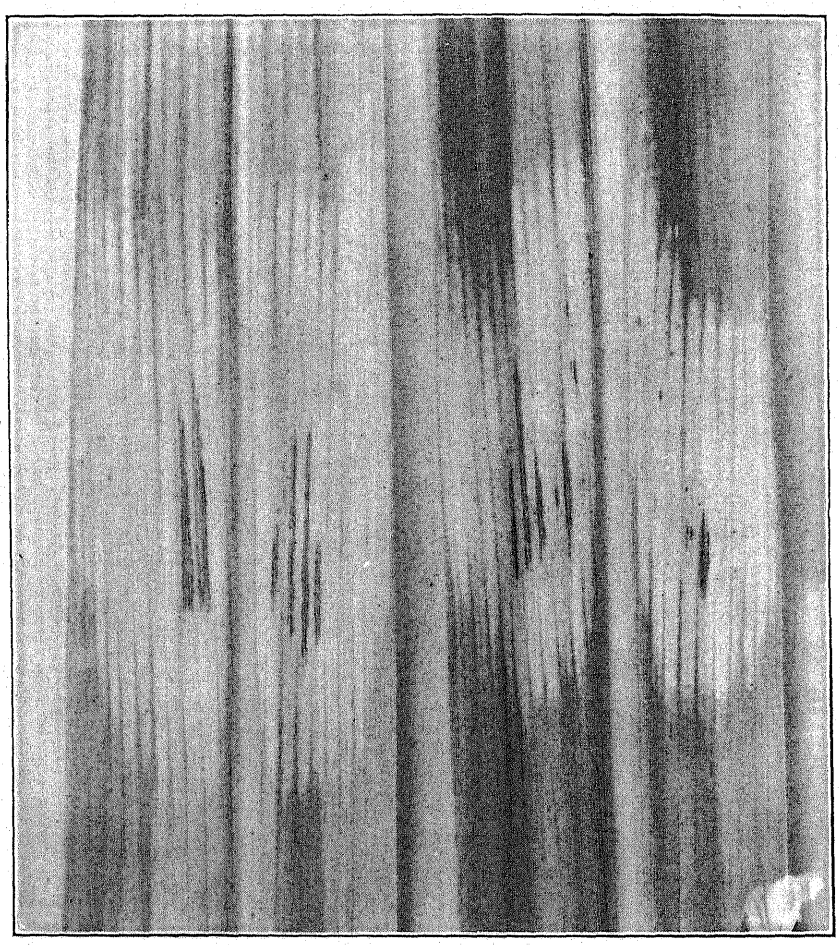

FIG. 3.—Cane leaves with chlorotic areas due to cold injury. The darkened stripes in the chlorotic area are due to the attacks of a weak fungus parasite. Natural size.

low temperatures have followed periods of rainfall. Usually winter rains in central Cuba follow the continental storms of the North and are, as a rule, followed by colder weather. Such conditions stimulate growth in the cane, and then the cold water standing in the cornucopia of the stalk chills the newly exposed tender tissues, thus causing the chlorosis in the leaves affected. This combination of conditions is shown graphically in figure 4, a chart showing the maximum and minimum temperature curves and rainfall at Baraguá from November 1, 1925, to March 7, 1926. The arrows indicate the date of first appearance of the white bands in the leaves of H-109 and D-1135 cane varieties. The chart also shows the dates of application

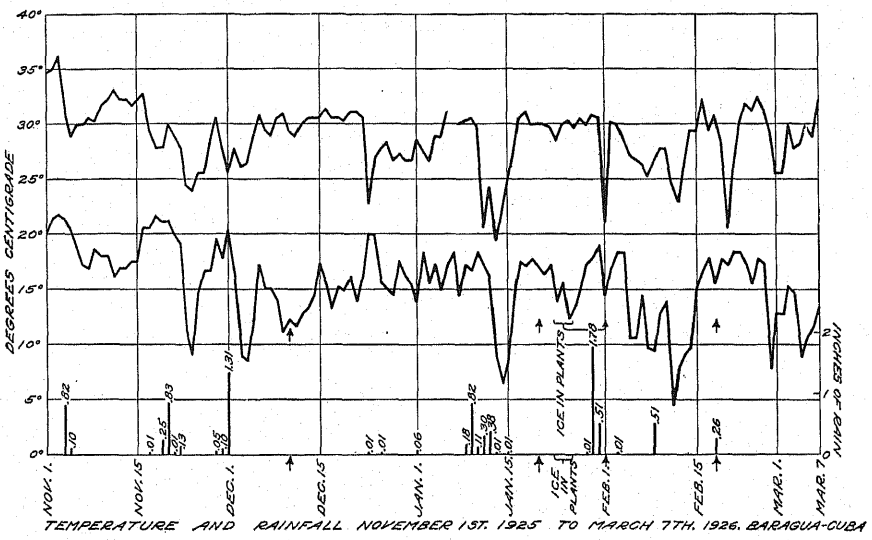

FIG. 4. Chart showing maximum and minimum temperatures and rainfall at Central Baraguá, Cuba, from November 1, 1925, to March 7, 1926. The arrows indicate the dates of appearance of cold chlorosis.

of the ice in the experiments and the appearance of the chlorotic bands thereafter.

From the chart it would seem that two or three nights of temperatures from 5° to 10° C. are required to produce this response in the susceptible varieties.

RELATIVE SENSITIVENESS OF CANE VARIETIES TO COLD CHLOROSIS

Some varieties are much more sensitive to this form of cold injury than others. This does not seem to be so much a matter of the age of the plants as the age of the tissues exposed and their inherent sensitiveness to cold.

Cold weather not preceded by rainfall to stimulate renewed growth may cause the appearance of slight chlorotic bands, while temperatures not quite so low but preceded by weather favorable for growth are followed by very marked chlorotic bands across the leaves. At the temperatures recorded in figure 4 the following varieties (Table 1) developed this form of white bands. The sensitiveness of the varieties is roughly indicated on the scale of 10, using 10 for the most sensitive and 1 for those varieties which developed slight chlorotic edges on the leaves.

Under the same general field conditions at Baraguá, Cuba, the following varieties did not develop these chlorotic bands in the leaves:

Badila
Cristalina
Striped Bourbon
Uba
Oshima
Burra
S.C.–12/4
Tucuman 439
B–3696
Cuba 1–34,
36–37, 40–45,
47, 49, 50, 52,
53, 64, 69, 145,
251, 291, 338,
350, 351, 362,
364, 384, 385,
390, 419, 483,
519, 555, 768
C.H.–64/21

Kavangire
Penang
Criolla
Toledo
Kinar
Banes 2
B–1753
Tucuman 507
B–6450
D–109
D–433
D–247
D–604
D–625
EK–28
M–36
B–208
Gomez Mena 4–6
do 8 and 9

La. Purple

Bocoya
Tekcha
Kassoer
Brava de Perico
B–1809
B–3412
B.H.–10/12
CO–210, 213,
214, 232, 281
P.O.J.–36, 213, 228,
234, 826, 979,
2379, 2714, 2221

Java Unknown
Caña Blanca
Tjepering
Bamboo Blanca
do Morada

B–3405
S.P.I.–33243
B–4596

Some of these varieties might develop this chlorosis at colder temperatures, or even at a greater prolongation of the temperatures recorded in the chart.

LEAF SPLITTING BY SECONDARY FUNGUS PARASITE

As shown in figure 3, when the leaves with these bands reach maturity, the tissues between the veins in some plants of certain varieties are attacked by a fungus which forms an elongated stripe. This stripe has a narrow reddish border and a gray center in which fruits of a Coniothyrium species are often formed. The leaves may split along these stripes, but the midrib and veins are usually strong enough to give mechanical support. The stripes occur on a very small proportion of the affected areas even in the susceptible varieties. They resemble very much the stripes formed upon chlorotic leaves of cane under injurious soil conditions such as areas of coco out-crops.

TABLE 1.—*Relative sensitiveness of cane varieties to cold chlorosis*

Variety	Sensitiveness to cold chlorosis	Variety	Sensitiveness to cold chlorosis	Variety	Sensitiveness to cold chlorosis
H–109	10	Kavangerie	3	B.H. 10/12	1
D–1135	9	Cuba 35	3	C–100	1
D–99	8	Cuba 483	2	D–116	1
D–74	5	P.R.–209	2	D–216	1
C–46	5	P.R.–707	2	F.C.–37	1
Yellow Caledonia	4	B–109	1	F.C.–306	1
		Cayana 10	1	P.O.J.–2725	1

SECTIONAL CHLOROSIS IN HAWAII

At the Honolulu meeting of the International Society of Sugar Cane Technologists,[2] the matter of sectional chlorosis of sugar cane was discussed. Its presence was reported in Australia by Mr. North, and in Hawaii by Mr. Lee. A similar trouble in Louisiana was reported by Mr. Brandes.

Mr. North suggested that the injury was associated with cold weather, as is the case with the cold chlorosis occurring in the fields in Cuba (Fig. 4). This is also confirmed by my experiments with ice in the spindle of the young cane plants. It is not necessary that the temperature should drop to freezing. In the more sensitive varieties temperatures around 10° C. seem to be sufficient to cause the injury. In Hawaii it has been noted that H–109 and D–1135 are very sensitive to the sectional chlorosis. This has also proved to be the case in the cold chlorosis herein reported (Table 1).

TROPICAL PLANT RESEARCH FOUNDATION,
CENTRAL BARAGUA, CUBA.

[2] Digest of the Proceedings of the 1st Conference of the International Society of Sugar Cane Technologists, Honolulu, 1924.

PHYTOPATHOLOGICAL NOTES

Virus mixtures that may not be detected in young tobacco plants.—For over a year the writer has used a virus of tobacco mosaic[1] which appears to be free from any other mosaic virus when observations are made on young plants grown at temperatures near 25° C. However, from three to six weeks after inoculations, small, inconspicuous yellow spots (1–5 mm. in diameter) begin to develop, usually on the older leaves. Rarely have more than three or four such spots been observed on a plant. At high temperatures (90°–100° C.) these yellow spots have been observed on 4 out of 400 young mosaic affected plants.

When young tobacco plants were inoculated with virus from these yellow spots and grown at 25° C., yellow mosaic developed in all the plants in from 10 to 12 days after inoculation. Later, green mosaic developed on the younger leaves, and this predominated over the yellow mosaic on the subsequent foliage. However, rather large, yellow spots eventually developed on the larger leaves of the infected plants.

When young plants were inoculated with virus from the green tissue bordering the yellow spots, a slight amount of yellow mosaic occurred in from 10 to 12 days, and later, green mosaic developed also; but when virus was taken from points on the same leaf more remote from the yellow spots, only green mosaic occurred on the young plants.

There are two points of outstanding interest in these results, (a) the suppression of yellow mosaic in the great majority of young plants when this virus is very dilute, and (b) the localized concentration of the yellow mosaic virus in the yellow areas.

These results make it apparent that a virus contamination may not be detected when the observations are confined to young plants. It seems evident that observations should be made for a considerable period after the occurrence of green mosaic in order to detect any possible outstanding variations in the symptoms. It now appears that the virus of the yellow mosaic has been purified by successive sub-transfers from the yellow spots.—H. H. McKinney, Pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Wisconsin Agricultural Experiment Station.

[1] This virus was obtained from a single plant which was kindly supplied the writer by Drs. James Johnson and M. Mulvania. The mosaic on this plant was of the typical green type which various workers have described.

Progress report on bunchy-top of abaca or Manila hemp.—Abaca or Manila hemp (*Musa textilis*) is one of the leading export crops of the Philippine Islands. Its most serious malady is bunchy-top, which is considered to be a virus disease. In all probability this is an old disease of abaca. The writer noted symptoms of it in the province of Albay, Island of Luzon, in 1910 and 1911. Dr. Otto A. Reinking brought a specimen from the same island from Silang, Cavite Province, on April 7, 1925. The alarming proportions of the disease in the abaca regions of the Philippines has recently attracted the attention of growers, and the writer's interest in bunchy-top was stimulated by a conference with Dr. Reinking.

The most striking characteristic of bunchy-top is the crowding of the leaves into a more or less rosette arrangement. Infected plants are much stunted and produce short pseudo-stems. Observations indicate that infected plants never recover. They die prematurely, and very few of them, if any, produce fruits. Abaca is susceptible to the disease at all times during its life cycle. Aphids were noted on bunchy-top infected abaca in Los Banos as early as June, 1925. On October 23, 1925, they were found in abundance on the unexpanded youngest leaves of healthy and bunchy-topped plants. This aphid has been identified as *Pentalonia nigronervosa* Coq. by Doctor L. B. Uichanco. It is of interest to note that in Queensland, Australia, bunchy-top of banana is reported to be transmitted from plant to plant by the same species.[1] We are led to believe that this aphid plays an important role in the transmission of bunchy-top of abaca in the field.

Results obtained from experiments using *Pentalonia nigronervosa* as vector indicate that bunchy-top may be transmitted from diseased abaca plants to healthy ones by this aphid. Material from several sources was used in the preliminary experiments, namely, young abaca plants grown in the Plant Pathology experimental plots, infected plants in the Agronomy field, and plants grown from seeds. Since bunchy-top plants do not produce fruits, disease-free plants were obtained from seed. Seeds were germinated in flats, and after emergence the seedlings were transplanted to pots where they were screened by cheesecloth. These seedlings were used in transmission experiments. Further work is in progress.—GERARDO OFFIMARIA OCFEMIA, Department of Plant Pathology, College of Agriculture, Los Banos, Philippine Islands.

[1] Anonymous. Bunchy top in bananas. Cause and nature of disease. Report of Investigation Committee. Queensland Agric. Jour. 24: 424–429. 1925.

PHOMOPSIS JUNIPEROVORA AND CLOSELY RELATED STRAINS ON CONIFERS

GLENN GARDNER HAHN

INTRODUCTION

The blight and canker of nursery cedars, caused by *Phomopsis juniperovora* Hahn (5) and known as cedar blight, probably should be regarded as a Phomopsis disease of conifers for reasons set forth in this paper. Stewart (16) and Duggar made the first recorded observations on this disease in 1896–7. Typical *P. juniperovora* continues to be in certain parts of the United States a virulent parasite, causing great damage in coniferous nurseries and also injury in ornamental plantings. It is now found to attack a number of heretofore unreported hosts which may be as vigorously attacked as is *Juniperus virginiana* L. (6), the host upon which red cedar blight, as the disease was earlier known (7), was investigated (4). Phomopsis strains closely related to *P. juniperovora,* but perhaps specifically distinct, have also been found to cause disease in representatives of a number of genera of conifers. The present paper is a progress report outlining the results of investigations in the United States.

GEOGRAPHICAL DISTRIBUTION

Typical *Phomopsis juniperovora* is known to occur in States extending along the Atlantic seaboard from Connecticut to Florida; from southern Minnesota and Wisconsin to Alabama; it is not known to date to extend any farther west than eastern Nebraska and Kansas (6). The States as heretofore published (5) include Illinois, Iowa, Kansas, Minnesota, Nebraska, New York, Ohio, and Pennsylvania. To this list may now be added Alabama, Connecticut, District of Columbia, Florida, Kentucky, Maryland, Missouri, North Carolina, Tennessee, and Wisconsin.

Closely related strains which have been found on conifers also are reported from California, District of Columbia, North Carolina, Pennsylvania, and Washington.

NEW FIELD HOSTS

Phomopsis juniperovora was originally described as occurring naturally on *Juniperus virginiana* (5) and *J. scopulorum* Sarg.[1] Early artificial in-

[1] The nomenclature herein followed is that contained in the revised check list of Commercially Important Trees of the United States, Forest Service, U. S. Dept. Agr., Dec. 15, 1924, and for ornamental conifers in Standardized Plant Names, American Joint Committee of Horticulture Nomenclature, 1924.

oculations (4) also demonstrated the potential parasitism of the *Phomopsis* on *J. virginiana, J. scopulorum, J. lucayana* Britt. (not *J. barbadensis* L.), *J. pachyphloea* Torr., *J. communis* L., *J. communis* var. *montana* (*J. sibirica* Burgsd.), *J. horizontalis* Moench (*J. prostrata* Pers.), *Thuja occidentalis* L., *T. orientalis* L., *T. plicata* Don., (*T. gigantea* Nutt.), *Cupressus glabra* Sudworth.

Diagnoses of field specimens sent into the laboratory have since shown that, with the exception of *J. pachyphloea,* all the above-mentioned inoculation hosts are naturally infected with *Phomopsis juniperovora.* Typical *P. juniperovora* has also been found on *J. procumbens* Sieb., (*J. chinensis* var. *procumbens* Endl.); *J. chinensis* var. *pfitzeriana* Spaett.; *J. sabina* L.; *J. sabina* var. *tamariscifolia* Ait.; *Cupressus sempervirens* L.; *C. macrocarpa* Hartw.; *C. arizonica* Greene; *C. goveniana* Gord.; *C. benthami* Carr.; *C. funebris* Endl.; *Chamaecyparis obtusa* Sieb. and Zucc., (*Retinospora obtusa* Sieb. and Zucc.); *C. pisifera* Sieb. and Zucc.; *C. pisifera* var. *plumosa* Beiss., (*Retinospora plumosa* Veitch); *C. lawsoniana* Parl., (*Cupressus lawsoniana* Murr.)

The Phomopsis strains related to *Phomopsis juniperovora* have been found on *Juniperus procumbens* Sieb.; *Thujopsis dolobrata* Sieb. and Zucc., (Hiba arbor-vitae); *Cephalotaxus drupacea* Sieb. and Zucc., (Japanese plum-yew); *Taxus baccata fastigiata* Loud., (Irish yew); *T. cuspidata* Sieb. and Zucc., (Japanese yew); *Taxodium distichum* (Linn.) Rich., (bald cypress); *Sequoia washingtoniana* (Winsl.) Sudw., (big tree); *S. sempervirens* (Lamb.) Endl., (redwood); *Cryptomeria japonica* D. Don, (common *Cryptomeria*); *Abies homolepis* Sieb. and Zucc., (*A. brachyphylla* Maxim, Nikko fir); *Pseudotsuga taxifolia* (Poir.) Britt., (Pacific coast type or Green Douglas fir, *Pseudotsuga douglasii* Carr., and the Rocky Mountain type, or Blue Douglas fir, *Pseudotsuga glauca* Mayr.); *Tsuga canadensis* (Linn.) Carr., (eastern or Canada hemlock); *Larix europaea* D. C. (*L. decidua* Mill., European larch); and *L. leptolepis* Murr. (Japanese larch).[2]

CULTURAL CHARACTERS

The separation of the Phomopsis strains into the two arbitrary groups, namely the A group, definitely typical *P. juniperovora,* and the B group, which is composed of the closely related strains, is based in part on cultural behavior in preliminary tests. Lines of the first-named group, which were isolated and studied from *Juniperus virginiana, Cupressus arizonica, C. sempervirens, C. funebris,* and *Chamaecyparis obtusa,* differed somewhat in their reactions from lines from the B group studied from *Juniperus pro-*

[2] It will be noted that the 22 host species of typical *Phomopsis juniperovora* all belong to four genera of cedars; while the related strains are mostly from other coniferous hosts.

JOSEPH ROSENBAUM

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H. H. Whetzel and I. E. Melhus

"He not only had a great many friends but he knew it too. He showed his affection for people so naively that one couldn't help returning it." No finer expression of the personal character of Joe Rosenbaum can be offered than these words of his stricken wife announcing the death of our young friend and colleague. We called him "Joe" because we sensed the human friendliness of him. We esteemed him for his enthusiasm and devotion in the labors of his profession. His researches in the field of plant pathology, to which the earlier years of his professional life were devoted, commanded our confidence and respect.

These characters of the man were already clearly defined in the days when, as a graduate student, he worked with the senior author in the laboratories of plant pathology at Cornell University. Keen and alert, he worked with the joyous enthusiasm, the clear thinking and the friendly devotion which every teacher recognizes as the mark of the promising student. With no trace of personal selfishness, which often mars the promises of a brilliant mind, he was an ideal assistant and companion in scientific endeavor. It was this rare combination of enthusiasm, personal charm and ability which during the later years of his short life made him a devoted and successful teacher of young men preparing to enter college. The last time I saw him he told me, with that generous pride of the successful teacher, of the high standing of the pupils from his laboratory in the entrance examinations in botany and biology. With undiminished enthusiasm and energy he had passed from the field of research to the more difficult and exacting realms of the successful teacher. And so, unsuspecting, I grasped his hand for the last time, and he left me as he had come to me in his college days, the kindly, able and enthusiastic Joe.

The junior author was fortunate in being associated with Joe as a fellow investigator when he served in the Bureau of Plant Industry, U. S. Department of Agriculture. In that association the sterling qualities of the man

gave greatest expression. He was ever ready to do his part in the fullest measure; and his enthusiasm, determination and untiring efforts, coupled with a keen analysis of his task, were stimulating and contributed much to lightening our labors in the field of potato disease investigations. His learning, alertness, openmindedness and honesty of scientific purpose made for implicit confidence in his interpretations and conclusions. In a word, his scientific contributions bear the stamp of a task well done.

The close contact of an isolated field station served to bring out those qualities of companionship so characteristic of the man and to emphasize the beautiful simplicity of his ideals in life. Here was shown his home life, presided over by his wife, a noble woman, radiating physical well-being, hope, and gladness in their path. Joe often spoke of his desire to have a small country place surrounded by plants in their natural beauty. His keen pleasure in serving others, his unselfishness, love of home simplicity, and his innate idealism are attributes that claim our homage.

Joseph Rosenbaum was born December 19, 1887, in Russia. He was descended from a long line of able Jewish Rabbis. He came with his father and the family to the United States, where they settled in Woodbine, New Jersey, in 1896. Here he received his grammar and high-school education. He entered the Massachusetts Agricultural College in 1906, transferring to the New York State College of Agriculture at Ithaca in 1907. He received the degree of B.S.A. from the latter institution in 1911. He supported himself while an undergraduate by writing and by miscellaneous work during summer vacation and through the school year. He received the degree of Doctor of Philosophy at Cornell University in 1914, having specialized in the field of plant pathology. During these graduate years he was special agent for the U. S. Department of Agriculture on ginseng diseases and, in 1914–1915, instructor in plant physiology in Cornell University. In 1915 he became a resident member of the staff of the Bureau of Plant Industry in the U. S. Department of Agriculture, where he remained, devoting himself to research in plant pathology and mycology, until 1920. From 1920 until the time of his death he was head of the Department of Botany and Biology at the Milford Preparatory School, Milford, Connecticut. Here he soon became a successful and much loved teacher. He died June 5, 1925, of bacterial endocarditis, after a three months' illness. He left a widow and two young daughters.

Dr. Rosenbaum was a member of the Alpha Chapter of Sigma Xi, a member and fellow of the American Association for the Advancement of Science, a member of the American Phytopathological Society, and of the Botanical Society of Washington, D. C. For one whose years in productive research were so few, he made a notable number of contributions to science.

PUBLICATIONS OF JOSEPH ROSENBAUM

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A Macrosporium foot-rot of tomato. Phytopath. 10: 415–422. 1920.

cumbens, Thujopsis dolobrata, Cephalotaxus drupacea, Taxus baccata fastigiata, Taxodium distichum, Sequoia sempervirens, Pseudotsuga taxifolia (both Pacific coast and Rocky Mountain types) and *Larix europaea.*

For the A group, tests were made with cultures from 15 localities in Maryland, District of Columbia, North Carolina, Illinois, and Missouri; 16 lines of this group were obtained from single spores. Cultures of the B group were likewise studied from 40 localities in Pennsylvania, District of Columbia, North Carolina, Illinois, Washington, and California; 45 lines were obtained from single spores.

Freshly isolated cultures of strains from the A group generally produce the characteristic empire-yellow to orange-rufous color and flaming orange crystals in the medium, both of which have been previously reported as diagnostic for *P. juniperovora* on prune or cornmeal agar (5). This orange color is also vividly produced in cornmeal plus 2 per cent cane sugar (saccharose) agar. Only one strain was found which produced it in glycerine agar. On Thaxter's medium, certain strains produced a wine color in the surface growth. On Molisch's medium the orange color appeared in the substratum, while the dark grayish surface growth had a delicate lilac cast with here and there shadings of green.

Growth of the strains from the B group on Thaxter's and Molisch's media, and on cornmeal, cornmeal plus 2 per cent cane sugar and glycerine agars, was unaccompanied by the production of the vivid orange-red color in the substratum described above. On Thaxter's medium a pale rose color appeared in the surface growth. On Molisch's medium the surface growth was indistinguishable from that of the cultures composing the A group. Growth in the B group generally was slightly more rapid, and pycnidia commenced to appear somewhat earlier than in the A group.

As the cultures of strains of both groups age, they become very much alike. Abundant pycnidiospores are produced by each group. The outstanding cultural characteristics which distinguished the two groups was the production of the orange-red color associated with the early growth of typical *Phomopsis juniperovora.* The production of this color also held for reisolations of strains of the A group from Douglas fir infected artificially with strains of *P. juniperovora* recently isolated from *Juniperus* and *Cupressus* from North Carolina and Maryland, respectively. Single spore cultures from red cedar were observed which produced only a bare suggestion of the color or lacked it entirely. These strains were classed as *P. juniperovora,* despite the abnormality in the color character, on the basis of their origin on a cedar host in a locality where the typical color-producing fungus was known to occur. Old cultures were observed to lose gradually their ability to reproduce the orange-red color. Continued investigation may demonstrate that color production is a variable characteristic. Heald

and Studhalter (9) found that different strains of *Strumella coryneoidea* Sacc. and Wint. in culture showed great variation in the amount and intensity of dark-brown or black coloration.

In line with the consideration of color in artificial culture as a variable factor, Bottomley (2), in culturing a highly parasitic *Phomopsis* causing blight of seedling *Cupressus* (*C. arizonica, C. macrocarpa,* and *C. torulosa* D. Don) in South Africa, did not obtain the orange-red coloration descriptive of cultures of *P. juniperovora* isolated from *Cupressus* spp. in the United States. She describes only a greyish-green color and the spores oozing from pycnidia in a pale salmon-pink drop.

MORPHOLOGICAL CHARACTERS

With regard to the size and shape of their spores, the closely related strains of *Phomopsis* differ only slightly from *P. juniperovora* as earlier described (5). The A and B groups both produced the two types of spores known as α and β spores respectively in the same or different pycnidia both naturally and under cultural conditions. The α spores were shorter, being usually oblong to ellipsoid, or spindle shaped with subacute extremities generally biguttulate; whereas the β spores were longer, filamentous, curved, sickle, S-shaped, or hooked at the upper end (5). The measurements of the α spores of the Transvaal fungus, as given by Bottomley (2), differ only very slightly in shape and size from *P. juniperovora.* The question therefore comes up as to what influence the particular host may have on the size of the pycnidiospores. While Shear, Stevens and Wilcox (15) found constant uniformity for the cultural characteristics and size of ascospores for *Physalospora malorum* and *Botryospheria ribes,* at the same time they admit fluctuation in size of pycnidiospores from different hosts. These writers call attention to Paddock's (10) observation with regard to the variation of the average size of spores of *Sphaeropsis malorum* produced on different hosts.

By reason of the slight differences between the two groups, the Phomopsis strains on conifers, other than those included in group A, may be tentatively regarded as strains of *P. juniperovora;* but, for the sake of clearness, the two groups are kept distinct throughout this report. An extensive cultural and morphological comparison between *P. juniperovora* and the related strains for the definite determination of the latter is now being undertaken, together with an effort to develop a perfect stage. These data will be reported later. The results of preliminary artificial inoculation experiments with strains from both groups are given in the following section.

NEW HOSTS ESTABLISHED BY ARTIFICAL INOCULATION

Cross inoculation experiments have indicated physiological relationships between strains of *Phomopsis juniperovora* and closely related strains of

the B group. Douglas fir, *Pseudotsuga taxifolia* commonly called Pacific Coast, Oregon or Green Douglas fir, has been shown (Table 1) to be a common host under artificial conditions, when inoculated with strains of *P. juniperovora* isolated from *Juniperus virginiana, Cupressus sempervirens, C. arizonica, Chamaecyparis obtusa,* and related strains isolated from *Taxus baccata fastigiata, Taxodium distichum,* and *Pseudotsuga taxifolia,* Pacific coast type. Results of cross inoculation of Phomopsis strains on various other hosts are presented in tables 2 and 3 and in the discussion.

METHODS OF INOCULATION

Artificial inoculations were made from April to July by wounding host plants from two to fourteen years old. At this time the plants were all growing actively. The parts to be inoculated were first thoroughly cleansed with a cotton swab and 95 per cent alcohol. A bit of a culture of *Phomopsis* in the pathological greenhouse where the plants were kept in an iceless bark with a sterile scalpel. The inoculations were protected by being wrapped with raffia and moist cotton. Some of the inoculations were made in the pathological greenhouse where the plants were kept in an iceless refrigerator[3] for 48 hours after inoculation; others were made in the plant beds out of doors. The latter received only the routine watering which is given the out-of-door plants. In all tests control wounds which were made on trees inoculated with the fungus or on uninoculated trees were treated as just described, with this exception—a bit of sterile cornmeal agar was inserted instead of the mycelium growing on agar.

RESULTS OF INOCULATIONS

Infections in young tissue as the result of successful inoculations with *Phomopsis juniperovora* were commonly found to show signs of spreading within five to ten days after the insertion of the inoculum. A typical terminal or lateral blight resulted. Infections obtained on laterals may extend down into the main axis. On stems of larger diameter, cankers formed as the result of the fungous extension, which grew slowly, generally involving only one side of the branch or main axis and killing one or more laterals on that side. Such body cankers which did not become confluent usually became more or less limited and tended to callus over. Cankers of this type showed a definite separating fissure extending into the inner bark along the periphery of the canker. Below this cracking of the cortical tissues callus tissue formed. The pathological aspects of the blight and canker as just described are very similar to those stated by Wilson (17, 18, 19, 20,

[3] Hunt, N. R. The iceless refrigerator as an inoculation chamber. Phytopath. **9**: 211–212. 1919.

TABLE 1.—*Results of inoculation experiments with strains of typical Phomopsis juniperovora and closely related strains on Pseudotsuga taxifolia*

Host	Age of host in yrs.	Location of plants	*Phomopsis juniperovora*		Inoculations		Controls		Age of wood in years at point of infection
			Strains of A group	Source of culture	Number	Percentage of infection	Number	Percentage of infection	
Pseudotsuga taxifolia. tree No. 4	8	greenhouse	*Juniperus virginiana* 41369 C	single spore	12	100			1, 2, 3, 5
do 6	8	do					9	0	
do 20	9	outside	do	do	6	83	2	0	3, 4, 5, 7
do 1	8	greenhouse	*Cupressus sempervirens* 41372 C	spore horn	6	100			2, 3, 4
do 2	8	do	do	do	9	100			1, 2, 3, 4, 5
do 3	8	do					6	0	
do 21	14	outside	do	do	11	100	5	0	2, 3, 4, 5, 6, 8, 9
do 6	8	greenhouse	*Cupressus arizonica* 41041 A	single spore	12	100	4	0	1, 2, 3, 4, 5
do 8	8	do	*Pseudotsuga taxifolia* 41048 A. Reisolation from inoc. with *Cupressus* 41041 A	inner bark	12	100	5	0	1, 2, 3, 4, 5, 7
do 11	8	do	*Chamaecyparis obtusa* 41376 B	do	9	100	2	0	1, 2, 3, 4
Totals (8 plants inoculated)					77	99	33	0	

TABLE 1.—(*Continued*)

Host	Age of host in yrs.	Location of plants	*Phomopsis juniperovora*		Inoculations		Controls		Age of wood in years at point of infection
			Strains of B group	Source of culture	Number	Percentage of infection	Number	Percentage of infection	
tree No. 7	8	greenhouse	*Taxus baccata fastigiata* 41035 A	spore horn	6	83	5	0	2, 4, 6
do 8	8	do					9	0	
do 12	8	do	*T. baccata fastigiata* 41037 A	single spore	11	46	5	0	1, 2, 3
do 13	8	do	*Taxodium distichum* 41040 A	do	10	50	4	0	2, 3, 4, 5, 6
do 10	8	do	*Pseudotsuga taxifolia* 41039 A	do	7	29	5	0	3, 5
do 22	14	outside	do	do	11	64	5	0	2, 3, 4, 5, 6
do 14	8	greenhouse	*P. taxifolia* 41045 A	do	12	17	4	0	5, 7
Totals (6 plants inoculated)					57	46	37	0	
TOTALS	3 trees inoculated and 3 checks outside				28	82	12	0	
	11 trees inoculated and 11 checks in greenhouse				106	74	58	0	

21, 22) and Alcock (1) for a serious Phomopsis disease on Douglas fir, hemlock, and Japanese larch in Great Britain which is attributed to *Phomopsis pseudotsugae* Wilson.

The fungus appears to spread most rapidly longitudinally in the inner bark, killing the cambium and staining the wood a brownish color. Very small stems, less than one-half inch in diameter, are quickly girdled, the parts beyond the point of inoculation dying more rapidly than parts below. The lateral extension of the parasite in stems of one-half or more inches in diameter is very much slower. In the latter instance the outer bark apparently offers considerable mechanical resistance to the inroads of the fungus.

The control wound in no case showed signs of infection but promptly healed over. An additional control was provided for the experiments by the inoculations of *Pestalozzia sp.*, a fungus which occurs commonly and abundantly on dead nursery cedars and conifers generally. Eight inoculations with the mycelium of *Pestalozzia sp.* made according to the method just described for *P. juniperovora* and related strains, and four controls made in the same manner on *Cupressus arizonica* remained free from disease. These results confirm those obtained by Bottomley (2) with a *Pestalozzia* sp. on the same host in Transvaal, South Africa.

The results of the inoculations on *Pseudotsuga taxifolia* are summarized in table 1. As has been stated above, Douglas fir is a common host for all the strains tested, namely those isolated from *Juniperus, Cupressus, Chamaecyparis, Taxus, Taxodium,* and *Pseudotsuga.* The symptoms of the disease produced, whether it was on young twigs or on older parts, was identical in all cases. The strains of typical *Phomopsis juniperovora* appear to be more virulent than the strains of the B group.

The foregoing inoculations on *Pseudotsuga taxifolia,* Pacific coast form, do not agree with the negative result published for *P. juniperovora* on this host in 1920 (5). This early negative inoculation consisted of a single test made in January in the greenhouse on a dormant tree of Douglas fir, of the Rocky Mountain type. This tree was the only one available for study at the time.

The results of inoculation experiments on two-year-old *Cupressus arizonica* are stated in table 2. The plants used in these experiments were exceptionally vigorous. Strains of *P. juniperovora* from *Juniperus, Cupressus,* and *Chamaecyparis* were extremely parasitic upon this host. Inoculation results with *P. juniperovora* isolated from *Cupressus* parallel those obtained by Bottomley (2) with the Transvaal *Phomopsis* upon the same host. Closely related Phomopsis strains from *Taxus* and Douglas fir produced negative results even when inoculated in very young tissue of *C. arizonica.*

The results of inoculation experiments with strains of the A and B groups on *Thuja orientalis* are stated in table 3. Strains from *Juniperus,*

Cupressus, and *Chamaecyparis* proved to be highly virulent on this host; Phomopsis strains from *Taxus* and Douglas fir failed to infect either very young or older tissues.

Three-year-old plants of *Taxodium distichum* were inoculated with strains of *Phomopsis juniperovora* from *Juniperus* and *Cupressus* (group A) and a Phomopsis strain from *Taxus* (group B). The strains of the A group were able to infect one- and two-year-old wood of this host; the infections on three-year old wood became limited and healed over. Of the 30 inoculations with strains of *P. juniperovora*, 22 were positive, while 6 inoculations with a Phomopsis strain from *Taxus* produced negative results. In all cases checks remained uninfected and promptly healed over.

A single three-year-old plant of *Larix europaea* inoculated with *Phomopsis juniperovora* showed infection within three days after inoculation.

TABLE 2.—*Results of inoculation experiments with strains of Phomopsis juniperovora and closely related strains on two-year old Cupressus arizonica in the greenhouse*

Host	No. of plants	*Phomopsis juniperovora*		Inoculations		Controls[a]	
		Strains of A group	Source of culture	Number	Per cent infected	Number	Per cent infected
Cupressus arizonica	3	*Juniperus virginiana* 41369C	single spore	11	100	4	0
	2	*Cupressus sempervirens* 41372C	spore horn	6	100	4	0
	2	*Chamaecyparis obtusa* 41376C	inner bark	6	100	4	0
	2	*P. taxifolia* 41048A. Reisolation from inoculation with *Cupressus* 41041A	inner bark	7	100	3	0
Totals	9			30	100	15	0
		Strains of B group					
Cupressus arizonica	3	*Taxus baccata fastigiata* 41035A	spore horn	9	0	3	0
	3	*T. baccata fastigiata* 41047A	single spore	9	0	4	0
	2	*Pseudotsuga taxifolia* 41045A	do	6	0	3	0
Totals	8			24	0	10	0

[a] Control inoculations were made on 7 separate plants.

All the inoculations on this plant were positive; and three checks on the same plant remained uninfected.

Negative inoculation results were obtained with strains of the A, or typical *Phomopsis juniperovora,* group on the following:

Juniperus stricta, 6 inoculations with a strain from *Juniperus* on 2 two-year- old plants.

Picea excelsa, 29 inoculations with a strain from *Juniperus* on 5 young plants; 8 inoculations with a strain from *Cupressus* on an eight-year-old plant.

Pinus radiata, 6 inoculations with a strain from *Juniperus* on 2 two-year-old plants; 4 inoculations with a strain from *Cupressus* on 2 two-year-old plants.

TABLE 3.—*Results of inoculation experiments with strains of Phomopsis juniperovora and closely related strains on two-year old Thuja orientalis in the greenhouse*

Host	No. of plants	*Phomopsis juniperovora*		Inoculations		Controls[a]	
		Strains of A group	Source of culture	Number	Per cent infected	Number	Per cent infected
Thuja orientalis	3	*Juniperus virginiana* 41369C	single spore	15	100	5	0
	4	*Cupressus sempervirens* 41372C	spore horn	15	100	10	0
	2	*Chamaecyparis obtusa* 41376C	inner bark	8	100	4	0
	2	*P. taxifolia* 41048A. Reisolation from inoculation with *Cupressus arizonica* 41041A	inner bark	12	100	4	0
Totals	11			50	100	23	0
		Strains of B group					
Thuja orientalis	3	*Taxus baccata fastigiata* 41035A	spore horn	9	0	4	0
	2	*T. baccata fastigiata* 41037A	single spore	8	0	3	0
	2	*Pseudotsuga taxifolia* 41045A	single spore	12	0	4	0
Totals	7			29	0	11	0

[a] Control inoculations were made on 8 separate plants.

P. strobus, 10 inoculations with a strain from *Juniperus* on 2 four-year-old plants; 14 inoculations with a strain from *Cupressus* on 3 three-year-old plants.

P. excelsa, 18 inoculations with a strain from *Juniperus* on 3 five-year-old plants.

P. sylvestris, 12 inoculations on the young growth of 2 nine-year old plants.

Negative inoculation results were also obtained with a strain of the B group from *Taxus* on *Picea excelsa.* Twenty-five inoculations on 4 young plants were negative.

The above-listed negative results for *Phomopsis juniperovora* on *Pinus* and *Picea* parallel other negative results (5) previously obtained on *Picea parryana* (Andre) Parry, *P. engelmanni* Engelm., *Pinus edulis* Engelm., *P. glabra* Walt., and *P. taeda* Linn.

FACTORS PREDISPOSING TO PHOMOPSIS ATTACK

Young coniferous nursery stock is virulently attacked by the cedar-blight organism, *Phomopsis juniperovora.* Field observations have shown that it is fully capable of entering and attacking thoroughly healthy trees unweakened by other causes. This ability to infect readily young, tender, unwounded tissue which has been demonstrated by Bottomley (2) in her experiments with a *Phomopsis sp.* helps to explain the appearance of typical cankers on older stock, caused by *P. juniperovora* and closely related Phomopsis strains getting into the older tissue of the main stem through the infected side laterals (Plate LII).

Contributory evidence of the parasitic ability of *P. juniperovora* as a nursery-seedling parasite has been brought out by Rathbun-Gravatt in her inoculation experiments with damping-off fungi. In one experiment she showed that *P. juniperovora* caused root-rot of pine seedlings (11). She not only found that this fungus caused considerable decay of coniferous tap roots (12), but also in direct inoculation of coniferous stems evidenced parasitic ability (13). *P. juniperovora,* out of six miscellaneous fungi tested, including *Pestalozzia sp.,* a species commonly associated with the *Phomopsis,* was the only one which caused any radicle decay. It also appeared to cause a decrease in sprouting (14). Hartley (8) and his associates in soil inoculations previously found that *P. juniperovora* did not cause damping-off of pine seedlings. The possible reason for this may be the inability of the fungus to maintain itself in the soil saprophytically until the seed has sprouted.

During growing seasons when excessive moisture is present the organism becomes particularly aggressive, blighting entire beds of seedling stock in a very short period of time. In one nursery where the *Phomopsis* has

caused heavy damage for many years it was found that overhead irrigation greatly increased loss due to the blight. A discontinuance of this system

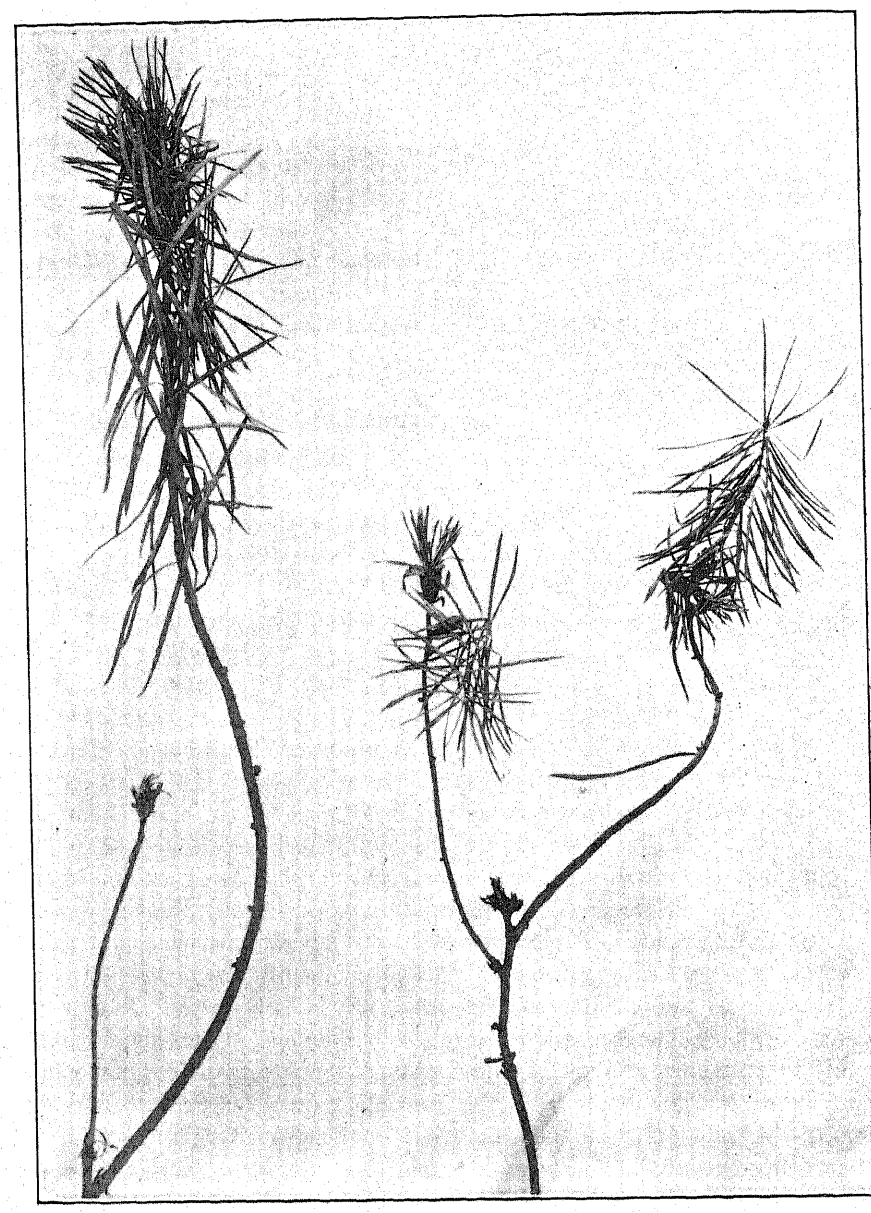

FIG. 1. *Pseudotsuga taxifolia*, Pacific Coast type (FP 41045) affected with a Phomopsis strain which closely resembles *Phomopsis juniperovora* and which causes a terminal and lateral blight of 3-year old nursery seedlings. Natural size.

of irrigation aided appreciably in the control of the disease. The crowding of plants in the seedling beds, and heavy shading also increased the toll of the disease, making possible moist chamber conditions for the abundant propagation of the fungus and rapid dissemination from plant to plant.

Since there is now abundant experimental evidence that infection takes place through wounds, injuries to the trees, no matter how produced, must be looked upon as predisposing factors. Transplanting, cultivating, pruning, in fact mechanical wounds of all sorts may lead to infection by the organism. It is not known how much insect injuries have to do with the spread of *P. juniperovora.* In the grafting process wherein scion stock of different horticultural varieties of cedars is used on rootstocks of *Juniperus virginiana,* heavy loss from the blight has been experienced, probably due to the fact that the fungus may enter through the graft union.

Undoubtedly the weakening of older trees by unfavorable growth conditions is likewise a factor for consideration in the spread of blight and canker. Ornamental trees, some of considerable age, have been found infected with *P. juniperovora* and strains of *Phomopsis.* Cankers from such trees placed in moist chamber produced Phomopsis spore tendrils in great abundance (Plate LIII).

EXTENT OF THE DAMAGE

Throughout the Middle West the growing of grafted and seedling nursery stock of *Juniperus virginiana,* especially the Platte River type, is, in certain seasons, seriously affected. A number of nurseries have been forced out of cedar growing by the blight. In Alabama the southern type of red cedar, *Juniperus lucayana,* is reported to be heavily infected. In coniferous nurseries in the South where conifer propagation is rapidly increasing, the successful propagation of ornamental cedars, particularly *Cupressus* and *Juniperus,* is extremely difficult during certain seasons because of damage by *Phomopsis.* Bottomley (2) has reported the devastating effect of a closely related Phomopsis blight in coniferous nurseries during certain years in Transvaal, South Africa.

The injury caused by *Phomopsis juniperovora* and the closely related strains commonly occurs in ornamental plantings in private gardens, plantings about public buildings, and city parks. The disease frequently produces an unsightly effect in these plantings due to the dead twigs and branches which appear among the healthy green ones. Evans (3) reports a dying-off disease of *Cupressus macrocarpa* hedges in South Africa due to a *Phomopsis sp.*

Two localities are known so far where strains of *Phomopsis* have affected naturally seedlings of Douglas fir, *Pseudotsuga taxifolia,* Pacific coast type, growing in nurseries, namely, North Carolina and Stabler, Wash-

ington. In North Carolina approximately 75 per cent loss occurred among three-year-old nursery stock. Tendrils of *Phomopsis* appeared abundantly upon the blighted plant parts. Microscopic examination showed both α and β spores present. The injury consisted of a blighting or death of the terminal growth (Fig. 1) or the entire plant was involved (Plate LIV), the disease apparently entering the main stem through a short lateral. Where the leader had been killed, a lateral usually took its place (Fig. 1). Infected seedlings were observed to be smaller than healthy ones of the same age. At Stabler, Wash., the blight affected both low and high altitude forms, apparently causing a greater damage among the former.

A Phomopsis strain was also found in Pennsylvania associated with a twig blight of 15-year-old Douglas fir, Rocky Mountain form. This damage was slight as compared with that observed in the infected nursery stock of the Pacific coast form just referred to. In the North Carolina locality it was noted that seedlings of the Rocky Mountain form growing close by the Pacific coast form were uninfected. Wilson (17,18) describes *P. pseudotsugae* as causing damage on the Green Douglas fir, *Pseudotsuga douglasii* Carr. (*P. taxifolia,* Pacific coast type). He also states that the disease has been occasionally found on *P. glauca* Mayr, Blue Douglas fir (*P. taxifolia,* Rocky Mountain type), and the variety *caesia* of *P. douglasii* (22). A recent communication from Dr. J. S. Boyce, who has made a survey of the Phomopsis disease of conifers caused by *P. pseudotsugae,* states, "*P. pseudotsugae* rarely attacks Blue Douglas fir in England or Scotland, which is also true on the Island of Bornholm in Denmark."

Our knowledge of the extent of the damage due to cedar blight in nurseries has in the past been somewhat confused due to the fact that disease caused by *Phomopsis* formerly has been attributed to frost, heat, drought, or transplant injury. Wilson (22) described a somewhat similar situation for the Phomopsis disease of conifers in the British Isles.

SUMMARY

Phomopsis juniperovora, the fungus causing cedar blight, is now known to be a widespread parasite in the United States causing serious damage in coniferous nurseries and ornamental plantings. Closely related Phomopsis strains have also been found which cause a blight and canker whose symptoms are similar to those of cedar blight. These strains, which have been placed arbitrarily in a B group, show certain cultural differences from the strains of the typical *P. juniperovora,* or A group. For reasons stated in the foregoing progress report the members of the B group may be regarded tentatively as strains of *P. juniperovora,* but for the sake of clearness they are considered separately. Twenty-two hosts, 12 of these new, are reported for the A group or *Phomopsis juniperovora,* together with 13 additional

hosts for the related strains. The A group contains the strains which are known to be definitely *Phomopsis juniperovora* isolated from the following genera: *Juniperus, Cupressus, Thuja,* and *Chamaecyparis;* the B group, those from *Juniperus, Thujopsis, Cephalotaxus, Taxus, Taxodium, Sequoia, Pseudotsuga,* and *Larix.* Host species of the above genera are listed together with species of *Abies, Cryptomeria,* and *Tsuga* on which *Phomopsis* was found but not studied in culture.

Preliminary spore measurements of strains of *Phomopsis juniperovora* and related Phomopsis strains show slight differences in the size and shape of their α and β spores, both of which are produced in abundance by both groups.

Host relationships for *Phomopsis juniperovora* and closely related strains have been established by preliminary artificial inoculations. Strains of both the A or typical *P. juniperovora* group and the related B group are parasitic on eight-year-old trees of *Pseudotsuga taxifolia,* Pacific coast form. Strains of the former group appear to be most virulent. Strains of the *P. juniperovora* group from *Juniperus, Cupressus,* and *Chamaecyparis* were also very parasitic on two-year-old stock of *Cupressus arizonica* and *Thuja orientalis*; negative results were obtained on these two last-named hosts with strains of the B group from *Taxus* and *Pseudotsuga.* Positive results were obtained on *Larix europaea* with *P. juniperovora* from *Juniperus.* Negative results were obtained on both *Pinus* spp. and *Picea excelsa* with strains of the two groups.

The predisposing factors to cedar blight and canker caused by *Phomopsis juniperovora* and to the similar blight and canker caused by the closely related Phomopsis strains are discussed, together with the extent of the damage associated with the disease.

OFFICE OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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DESCRIPTION OF PLATES

PLATE LII

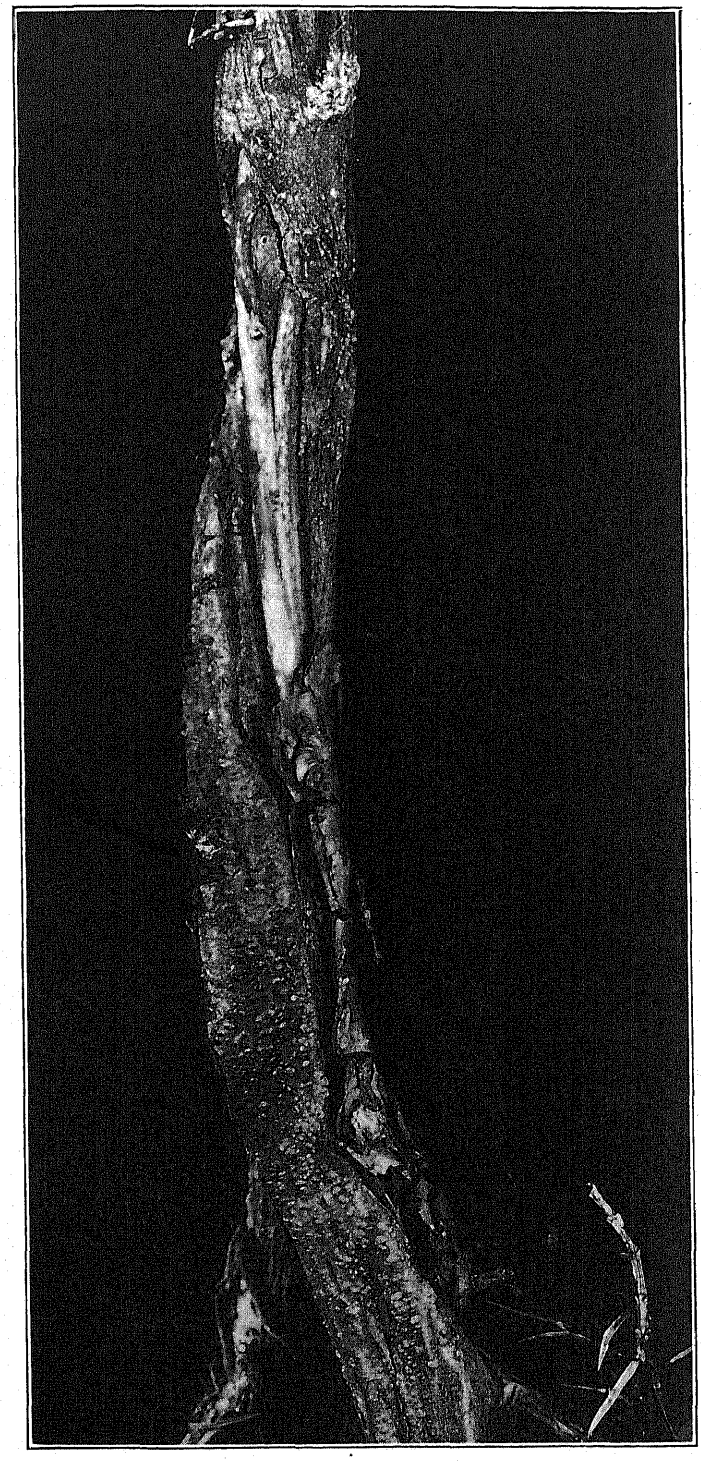
Juniperus virginiana affected with *Phomopsis juniperovora* (FP 41046). The canker on a 2-year old nursery seedling has girdled the main stem. Pustules abundant. Approximately 1½ times natural size.

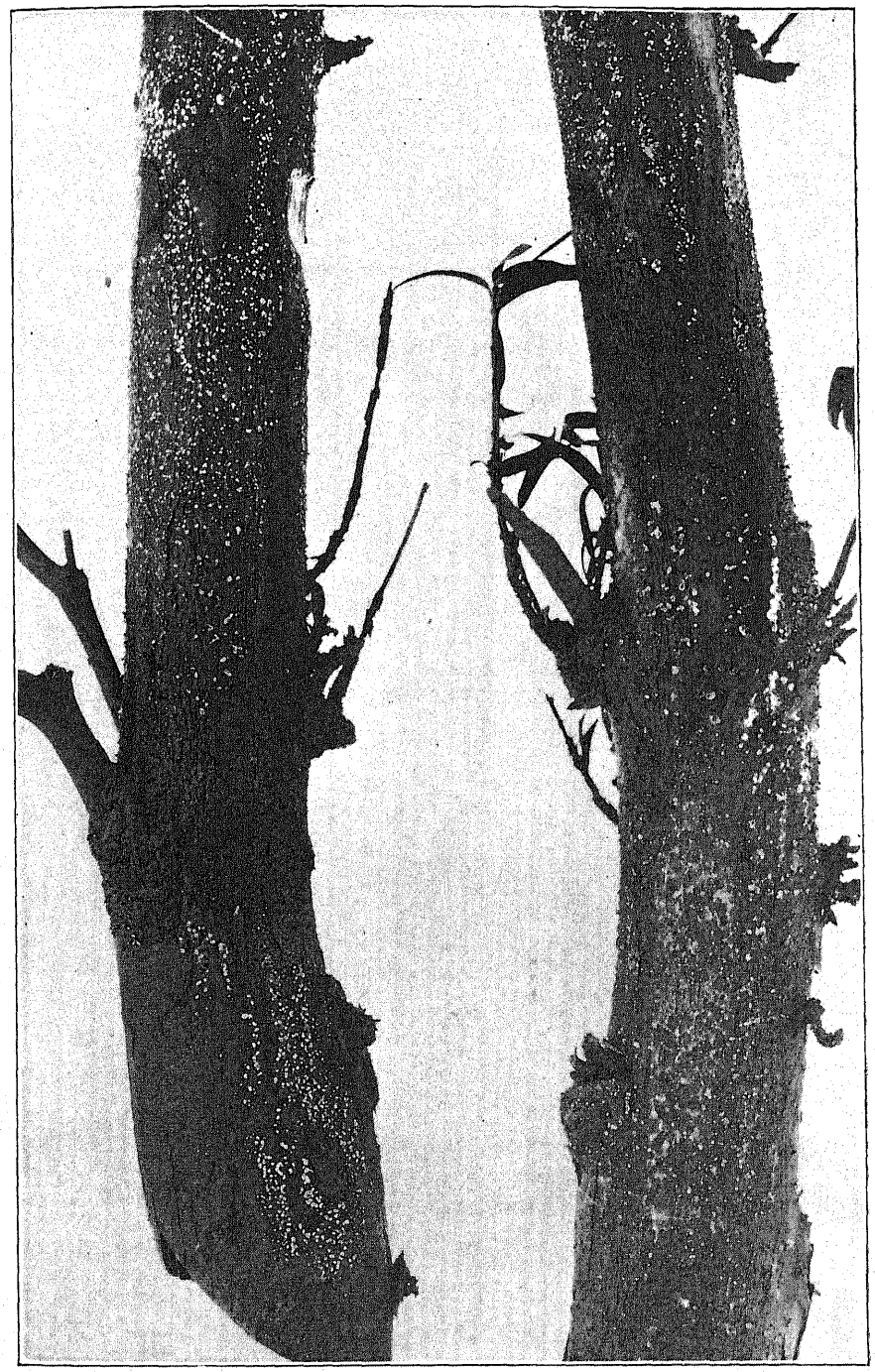
PLATE LIII

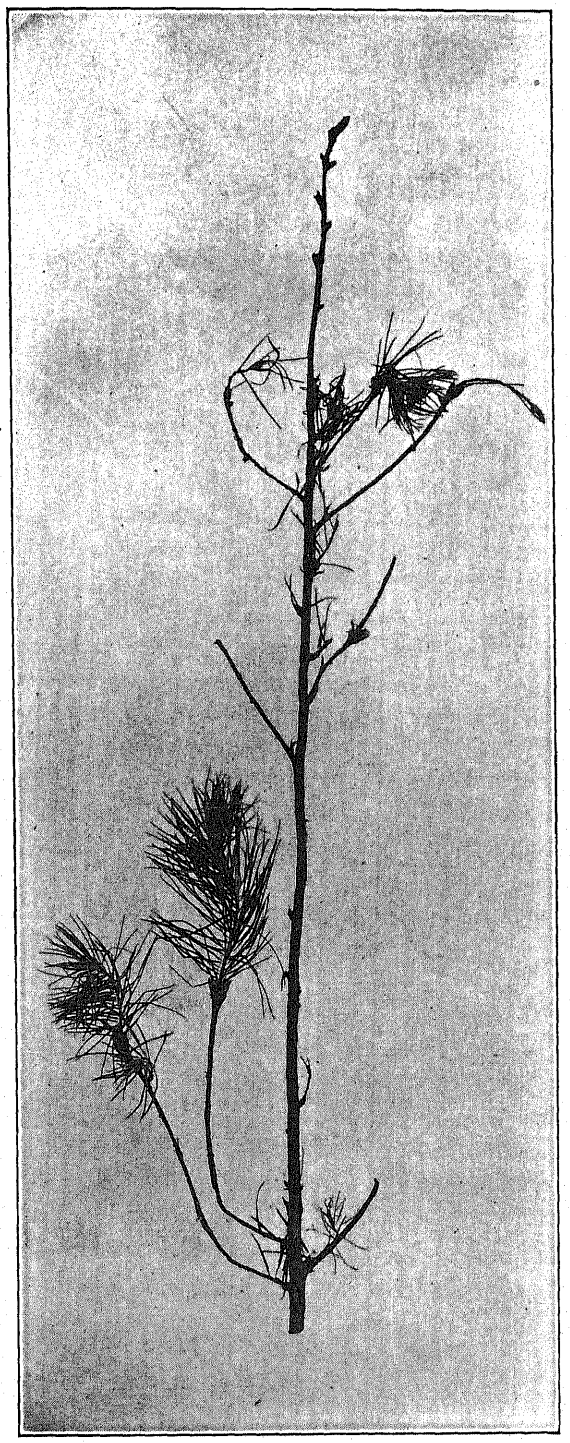
Phomopsis strain closely resembling *P. juniperovora* on *Taxus baccata fastigiata* (FP 41035). Moist chambered limbs of large infected tree showing spore tendrils of *Phomopsis*. Natural size.

PLATE LIV

Pseudotsuga taxifolia, Pacific Coast type (FP 41045) affected with a Phomopsis strain which closely resembles *Phomopsis juniperovora*. The entire terminal down to the base of the 3-year old nursery seedling was blighted. Spore tendrils of *Phomopsis* appeared on the stems in moist chamber. ½ natural size.







A NEW BACTERIAL DISEASE OF THE BEAN

WALTER H. BURKHOLDER

During the last few years in New York State the diseases[1] of bean (*Phaseolus vulgaris* L.) commonly referred to as the bacterial blights have been very severe, and have been the chief limiting factor in the production of such varieties as the Red Kidney. Many acres of this variety have gone unharvested owing to the ravages of these diseases. That the severity and the rapid spread of the bacterial blights during recent years were due to peculiar weather conditions seemed scarcely feasible. It was thought formerly, and with very good reason, that the blights were favored by hot weather. The weather in New York State during these recent severe epiphytotics has been varied but, for the most part, cool. That certain very virulent strains of *Phytomonas phaseoli* and *Phyt. flaccumfaciens* occurred and had spread over the State did seem possible. For this reason, late in the season of 1924 many specimens of the disease were collected from various parts of the State, and isolations of the causal organism attempted. Some surprise was experienced when, in many cases, it was impossible to obtain a yellow bacterium from badly diseased plants. However, a pale, colorless to white bacterium always appeared in these instances, and not infrequently was found associated with *Phyt. phaseoli* and *Phyt. flaccumfaciens*. The first few plates of the organism were discarded as contaminated, but, upon continued isolation, inoculation experiments were decided upon. These first experiments on some 16 Red Kidney plants immediately proved the organism to be pathogenic and quite virulent. This led to a careful observation of the disease in the field and in the greenhouse, and to a study of the pathogene in the laboratory.

SYMPTOMS

The symptoms produced on the bean plant by the pathogene under discussion are in the majority of cases not dissimilar to those produced by *Phyt. phaseoli* and *Phyt. flaccumfaciens*. In fact it is frequently impossible to distinguish the three diseases in the field. For this reason it has appeared best, when talking to growers, to use the term bacterial blight to cover all these troubles of the crop. The symptoms of this new disease are as follows.

[1] *Phytomonas phaseoli* E. F. S. and *Phyt. flaccumfaciens* Hedges cause similar diseases of the bean in New York State.

Wilt. A wilt of a portion or of the entire plant may occur, similar to that described by the writer[2] due to *Phyt. phaseoli* and by Hedges[3] due to *Phyt. flaccumfaciens.* The leaves wilt, turn brown and cling to the plant. Young pods may wither and fail to produce seed. In young plants the stalk dries, becomes moderately thin and topples over. When seedlings are artificially inoculated in the greenhouse by pricking the stem, a wilt and death of the entire plant may ensue within two weeks. This seedling wilt, however, has seldom been observed in the field. A wilt of the mature plant in the field, on the other hand, is one of the commonest symptoms. The disease in this form may spread over an entire field of Red Kidneys in a very short time. This wilt, as well as that caused by the other organisms, is the result of the pathogene infecting the vascular system.

At other times when the bacteria have gained entrance to the vessels and conditions are not favorable for a wilting to occur, a distinct dwarfing of the plant takes place.

On the leaves. Probably the most characteristic symptom of this bacterial disease occurs on the leaves of plants affected with the dwarfing described above, or on other systemically infected plants showing slight wilting symptoms. The younger and top leaves of the plant become mottled with pale and dark green areas (Fig. 1). As a rule the darker areas are closer to the large veins, and the lighter green areas are between. A crinkling of the leaf also occurs. This condition may cover an entire leaf or take place to a less degree in spots. This mottling has been found frequently in Red Kidney fields and has been mistaken by growers for mosaic, which at times it closely resembles. On the White Marrow this symptom even more closely resembles mosaic, but can usually be distinguished by the presence of other symptoms of this bacterial disease.

Necrotic lesions also occur on the bean leaves (Fig. 2). These spots vary in size, from one-half inch in diameter to areas covering the entire leaflet. They may be green and wilted at first, but later dry out and become brown. Frequently they have a light yellow-green border. The writer has been unable to distinguish them from similar spots caused by *Phyt. phaseoli* and *Phyt. flaccumfaciens.*

On the stems. Reddish, necrotic lesions extending longitudinally along the stem have been observed on plants affected with this disease. These lesions may be due to local infections, or may develop from the bacteria breaking through to the surface in vascular infections. In the latter case, small gray viscid drops have been observed on the lesions. Upon micro-

[2] Burkholder, Walter H. The bacterial blight of the bean: a systemic disease. Phytopath. 11: 61–69. 1921.

[3] Hedges, Florence. Bacterial wilt of beans (*Bacterium flaccumfaciens* Hedges) including comparisons with *Bacterium phaseoli.* Phytopath. 16: 1–22. 1926.

scopic examination these have been found to be masses of bacteria, and are not unlike those occurring on fire blight lesions of apple and pear trees.

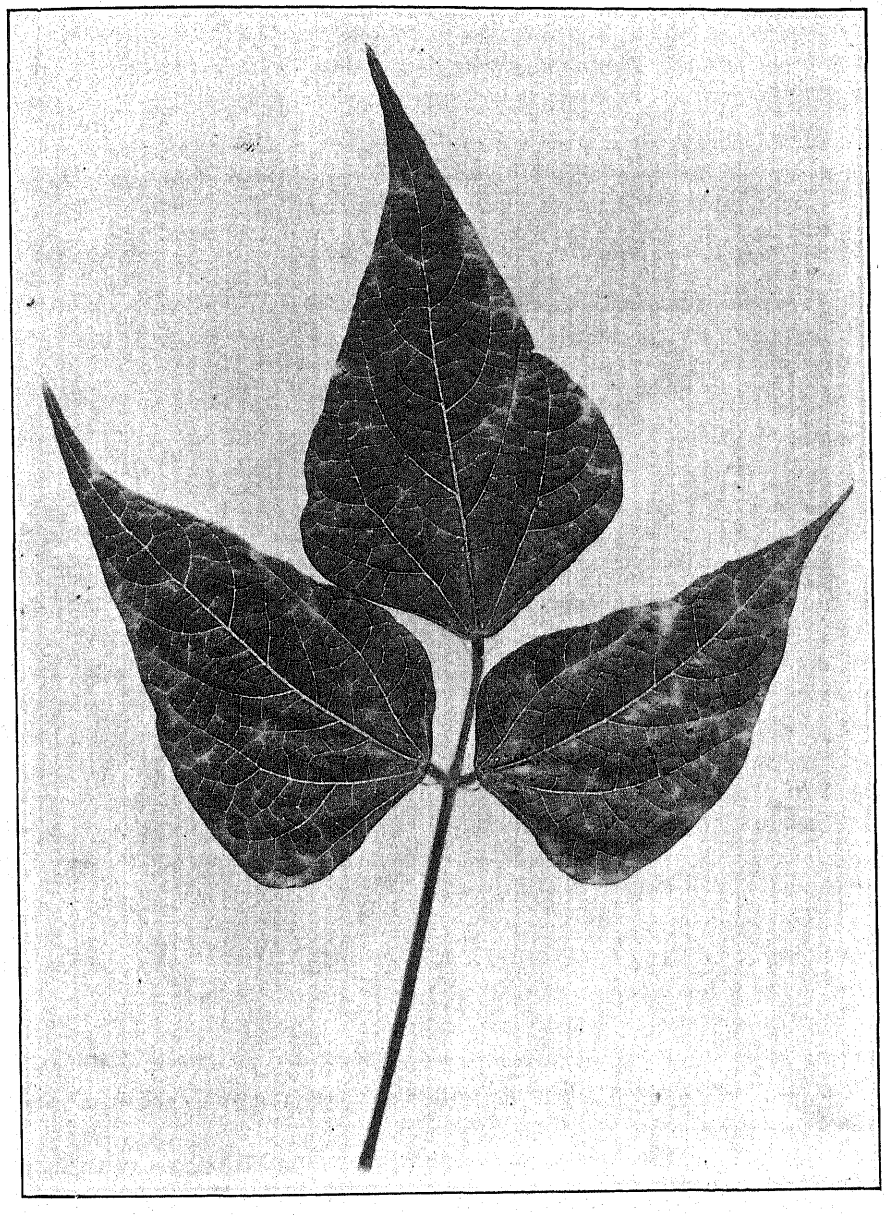


FIG. 1. Leaf of a White Marrow plant showing mottling due to the organism in the vascular system.

When bean plants are inoculated at the cotyledon or leaf nodes by the introduction of the pathogene on the point of a needle, a stem girdle not infrequently results. In appearance this symptom is identical with that due to *Phyt. phaseoli.* No work has been done in the field to determine the extent or frequency of the stem girdle due to this recently isolated bacterium.

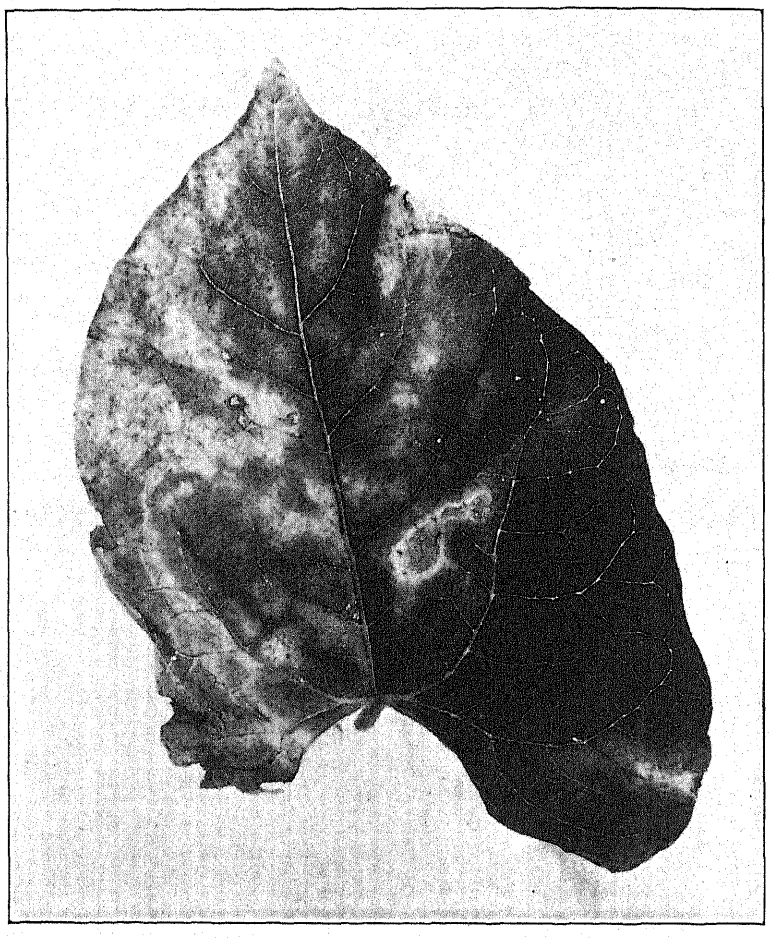


FIG. 2. Primary leaf of a bean plant which was inoculated at the cotyledon node.

On the pods. The lesions on the pods are not unlike those caused by *Phyt. phaseoli* (Pl. LV, A). The greatest difference is that they seem to spread more rapidly in the field and are probably more water-soaked in appearance. When they dry out it is very difficult to distinguish between

the two. Individual infections are more or less round, but they anastomose and upon drying become irregular in shape, and brick red to brown. The spots caused by *Phyt. phaseoli* frequently have a slight, yellowish incrustation covering them, while those due to the white organism have a silvery incrustation. It should be stated, however, that the incrustation in both diseases occurs on the larger and more severe lesions and then are not long lived. They are easily brushed off. Consequently this character can seldom be used to distinguish between the two diseases.

On the seed. Very small spots may occur on the seeds, or the entire seed may be practically destroyed. On colored seed it is impossible to distinguish the new disease from the other bacterial diseases of the bean or from the small spots due to the fungous pathogene, *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. et C. On white seeds the distinguishing characteristic is the maize yellow to cream color[4] of affected areas. Seeds severely infected are small and wrinkled and may be completely colored by the pathogene.

The bacteria may enter the seed either through the pod or through the vascular system. The pathogene undoubtedly overwinters on the seed as do the causal organisms of the other two bacterial diseases of the bean.

ISOLATION

The organism causing this disease has been isolated a number of times from specimens collected in various parts of New York State. It has been obtained from infected seeds, from pod spots, from lesions on the leaves, and from the interior of stems of systemically infected plants. Where there are visible necrotic symptoms of the disease on the plant, the bacteria have always been in abundance. Maceration of small portions of lesions in sterilized water and the use of loops full of this water in dilution plates has always yielded the organism.

In studying the pathogene the writer used three cultures isolated from various places in New York State. Two came from Wyoming County, one isolated from a Perry Marrow plant and one from a Wells' Red Kidney plant. The third culture was isolated from a Red Kidney plant collected in Wayne County. No variation was observed in the three.

MORPHOLOGY

The pathogene is a small rod with rounded ends, occurring singly or in pairs. On beef-extract agar which is somewhat acid long filaments are formed (Pl. LV, C). The organism stains readily with Ziehl's carbol

[4] Ridgeway, R. Color standards and color nomenclature. 44 pp., 53 col. pl. Washington, D. C. 1912.

fuchsin, gentian violet, and Loeffler's methylene blue. With all stains the contents of the organism appear to be granular, and very frequently a definite bipolar staining is obtained. From a 24-hour culture on beef-extract agar (pH 6.9) the rods stained with carbol fuchsin measure 1 to 3 μ by 0.5 to 1.25 μ.

The organism is actively motile, and on beef-extract agar one single polar flagellum has been demonstrated. Capsules have not been found.

CULTURAL CHARACTERISTICS

All cultures were kept in an incubator at 27° C. unless otherwise noted.

Beef-extract agar stroke (pH 7). Growth is scanty to moderate appearing in about 24 hours, colorless to white, at times creamy, filiform, with smooth, glistening surface, translucent. The consistency is at first butyrous, but becomes brittle with age. The medium remains unchanged. No odor detected.

Poured agar plates. The surface colonies develop at a moderate rate, becoming circular and glistening with a zonation about the edge. They are somewhat colorless to white and translucent. Their peculiarities may be observed in Plate LV, D.

Beef extract gelatin stab (pH 7). Kept at 20°–21° C. for six weeks. No liquefaction. A good white surface growth was formed, and a feathery, arborescent growth along line of puncture.

Poured gelatin plates. Colonies are slow growing, circular, raised, and slightly wrinkled. They are not very characteristic.

Beef extract bouillon (pH 7.1). In beef extract bouillon the optimum growth is reached at the end of 24 hours. The medium becomes cloudy, and a delicate pellicle is formed which breaks up if disturbed. A light precipitate is produced.

Litmus milk. A bluish ring appears at the surface of the milk in two to three days, and the color gradually extends throughout the medium. At the end of two weeks there appears to be a slight reduction of the litmus at the bottom of the tube. This whitens somewhat and then turns muddy, the top half remaining blue. At the end of six weeks no further change takes place.

Potato cylinders. A very scant growth barely visible to the eye is produced. Starch is not digested, but the medium is somewhat darkened.

Starch agar plates. When tested with iodin solution at the end of 7 and 14 days no digestion was observed.

Uschinsky's solution. A white turbid growth at first, soon becoming yellow green. Color appears first in a layer at the surface of the medium and gradually extends to the bottom. Pellicle formed which breaks up, leaving a heavy sediment.

Fermi's solution. In 24 hours, growth becomes visible. The medium later becomes cloudy and very slightly green in color. A heavy pellicle forms, which, upon being disturbed, falls to the bottom.

Cohn's solution. No visible growth.

Dextrose broth.[5] In fermentation tubes the medium becomes slightly cloudy in 24 hours. A heavy pellicle is then formed; the growth in the open arm becomes very cloudy and gradually extends into the closed arm. Acid is noted on the second day but a reaction shortly sets in and the medium becomes alkaline. No gas is produced.

Saccharose broth. In the open arm of a fermentation tube growth begins in about 24 hours. The medium is slightly cloudy at first with a light pellicle, progressing until it is turbid with a heavy pellicle. A good cloudy condition extends throughout into the closed arm. On the second day a slight acidity may be detected, but the medium shortly becomes alkaline again. No gas is produced.

Lactose broth. Growth begins in approximately 24 hours, and the medium becomes cloudy with a light pellicle. There is no clouding of the closed arm, and no acid or gas is produced.

Maltose broth. A cloudy growth appears in the bowl at the end of 24 hours. Later a light pellicle is formed. Acid or gas is not produced.

Nitrate reduction. Nitrates are not reduced. The organism was grown in a nitrate bouillon and test made at the end of 4 and 10 days with sulphanilic acid and alpha naphthalamine in 5N acetic acid.

Hydrogen sulphid production. Stabs were made in a lead acetate agar but no blackening or browning of the medium occurred along the line of inoculation. Positive results were obtained on this agar with *Bact. coli.*

Indol production. Indol was not produced. The medium used was Dunham's solution which had been prepared with a pre-war Witte peptone. The Ehrlich test was employed.

Oxygen relations. The organism grew well in the closed arm of fermentation tubes in both dextrose and saccharose broth. In shake agar cultures of ordinary beef extract agar, colonies were formed 3.5 cm. below the surface.

Longevity. The organism has lived for 12 months on beef extract agar kept at a temperature of 3°–6° C. Its virulence has not been impaired.

Thermal death point tests. Test tubes of a uniform thickness and containing 10 cc. of a beef-extract broth were used in these experiments. Two

[5] The sugar broths used here were the dehydrated broths obtained from the Digestive Ferments Co. They were the ordinary beef-extract broth to which the sugar to be tested and Andrade indicator had been added. After sterilization the reaction varied with the lot of media prepared from pH 7.3 to pH 7.6.

lots of broth were used at various times having a pH value of 6.9 and 7.0. To these tubes were transferred two large loops full of a 24-hour broth culture, and the tubes were immersed in a water bath for 10 minutes. The water was kept agitated and accurate temperature readings were made continuously with a certified thermometer. From a number of such experiments it was determined that the thermal death point was approximately 49° C.

NOMENCLATURE

If the above descriptions of the morphology and cultural characters of this organism are examined, it will be noted that they agree remarkably well with the descriptions of *Phyt. medicaginis* Sackett.[6] Other closely related organisms are *Phyt. sojae* Wolf, *Phyt. glycineum* Coerper and *Phyt. trifoliorum* Jones *et al.* Certain minor differences, however, may be found between the alfalfa organism and the bean organism, notably in the reaction of the pathogenes on several of the various media used. These are recorded in table 1. It must be admitted that these differences are slight and are ones which should scarcely be used as specific. In the first few cases they

TABLE 1.—*Differences noted between Phyt. medicaginis and the bean pathogene, Phyt. medicaginis var. phaseolicola*

Phyt. medicaginis	*Phyt. medicaginis* var. *phaseolicola*
Flagella 1–4, bipolar	Flagellum 1, polar
Nutrient agar becomes fluorescent	No change noted
No acid produced in dextrose broth	Slight acid produced in dextrose broth
No acid produced in saccharose broth	Slight acid produced in saccharose broth
No growth in closed arm of fermentation tubes in the above broths	Growth in closed arm of fermentation tubes in the above broths
Pathogenic to alfalfa	No infection obtained on alfalfa

even might be attributed to differences in technique or interpretation. When the matter was placed before Dr. W. G. Sackett, he was inclined to consider this organism a variety of *Phyt. medicaginis.* It should be stated here, however, that there was no culture of the alfalfa organism obtainable for comparison. The differences listed in table 1, especially the difference in pathogenicity, nevertheless appear sufficiently great to be considered varietal. For these reasons the bean organism is treated as a variety, and the name *phaseolicola* is given to it.

[6] Sackett, Walter G. A bacterial disease of alfalfa. Col. Agr. Exp. Sta. Bul. 158: 1–32. 1910.

TECHNICAL DESCRIPTION

Phytomonas medicaginis var. **phaseolicola** n. var.

Non-spore-forming rods with rounded ends; occurring singly or in pairs; cells 1.5 to 3 μ by 0.5 to 1.25 μ; on some media forming long filaments; motile by means of one polar flagellum; Gram negative, and not acid fast; when stained with carbol fuchsin or methylene blue showing more deeply stained areas which frequently are bipolar; a facultative anaerobe.

Colonies on nutrient agar, white to colorless, or at times, creamy, slow to moderate growing, concentrically ringed with edges undulate; gelatin colonies, slow growing, circular, raised, and somewhat wrinkled; agar streak, a moderate growth, colorless to white, flat, glistening, translucent, butyrous but later brittle; in nutrient broth a cloudy growth within 24 hours and pellicle formed later; litmus milk becomes an intense blue, but no curd formation nor peptonization occurs; produces a small amount of acid in dextrose and saccharose, but reverts almost immediately to the alkaline side; no acid in lactose or maltose; no gas formed in the above sugars; produces neither nitrite nor gas in nitrate media; does not liquefy gelatin, nor digest starch; no production of indol in Dunham's solution; no hydrogen sulfide produced in basic lead acetate agar; in Uschinsky's solution, a light yellow green turbid growth; a slight trace of green in Fermi's solution; no growth in Cohn's solution; thermal death point approximately 49° C.

Pathogenic on the common bean (*Phaseolus vulgaris* L.), the lima bean (*P. lunatus* L.), and the scarlet runner bean (*P. multiflorus* L.).

INOCULATION EXPERIMENTS

In conducting inoculation experiments several procedures were used, the most usual of which was the introduction of the pathogene into the stem by pricking with a needle or sharp-pointed scalpel carrying the bacteria. These wounds were inflicted either at the cotyledon or first leaf node. When conducting these experiments in the greenhouse no further care of the plants was necessary; that is, they were not placed under excessively moist conditions for the pathogene to establish itself. Several hundreds of such experiments were conducted during the last year and a half, and in every case excellent infection was obtained, except where varieties more or less resistant were used.

When Red Kidney seedlings were taken as the host plant and the experiments were conducted in a greenhouse held at approximately 80° F., the incubation period was about four days. In some of the cooler houses a week or so was required for the first symptoms of the disease to appear, and, if more mature plants were used, the time was still longer. Good infection,

however, occurred at 65° F., while infection at this temperature with *Phyt. phaseoli* was exceedingly light, and but moderate with *Phyt. flaccumfaciens.*

When bean pods were inoculated by pricking with a needle bearing the pathogene, distinct water-soaked areas about the point of puncture could be detected readily within three days. On young pods these lesions spread rapidly and not infrequently involved considerable tissue. A high temperature was maintained throughout these experiments.

That the pathogene requires a wound through which to enter the plant seems to be indicated by the following experiments. In one group of experiments 12 Red Kidney seedlings were used, and in another group 12 Red Kidney seedlings and 5 Robust pea bean seedlings. These were sprayed with a water suspension of the pathogene and held under moist conditions over night. In each case several Red Kidney seedlings were inoculated by pricking them at the cotyledon node with needles which had been smeared with a culture of the pathogene. These latter became infected, but in no case did one of the sprayed plants show symptoms of the disease. At another time six Red Kidney pods were painted with a water suspension of the bacteria and two of them pricked with a needle. The disease quickly appeared on the wounded pods, but the uninjured pods remained healthy. Further experiments, however, should be conducted before coming to any definite conclusions on this point.

VARIETAL SUSCEPTIBILITY

While no extensive experiments have been conducted to determine the varietal susceptibility of beans to this disease, yet certain observations have been made which should be reported. The Red Kidney, including the Wells' strain, is without doubt the most susceptible. Whole fields of this variety have been destroyed; and when seedlings are artificially inoculated in the greenhouse, over 75 per cent of them die. The White Kidney, too, is probably equally susceptible. The White Marrow and the Pea bean are somewhat more resistant, although they should never be recommended as resistant varieties. Inoculations in the greenhouse do not, as a rule, completely kill them.

The results of the following experiments indicate that practical resistance may be found for this disease. Nine seedlings of the White Kidney variety, 15 of Scotia, 16 of Refugee or Thousand-to-one, 6 of the Dwarf Grenell Rust Proof Wax, and 14 of London Horticultural were inoculated by pricking at the first leaf node. At the end of a week all showed unmistakable symptoms of the disease with the exception of Refugee and Scotia. One or two plants of these latter varieties finally showed faint symptoms, but all the plants grew to maturity and produced very satisfactory yields.

CROSS INOCULATIONS

A great many inoculation experiments were conducted with *Phyt. medicaginis* var. *phaseolicola* on a number of species of plants, mostly legumes, to determine, if possible, other hosts for the pathogene. To be sure that in all experiments a virulent culture of the organism was being used, Red

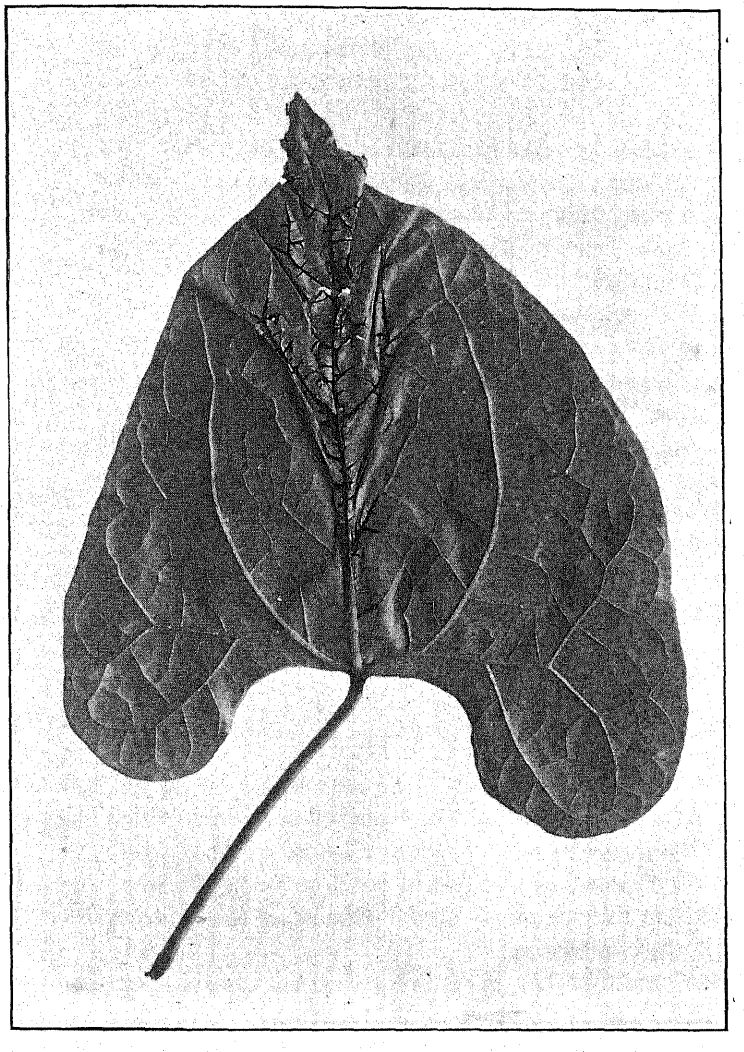


FIG. 3. Leaf of a lima bean plant showing killing of the veins due to *Phyt. medicaginis* var. *phaseolicola* infecting the vascular system. The plant was inoculated artificially at a lower node on the stem.

Kidney bean seedlings were inoculated and held under conditions similar to those under which the other plants were being tested. Without exception these check plants became infected. However, only two other species were found susceptible to this pathogene: these were of the genus *Phaseolus*—the lima bean (*P. lunatus* L.), and the scarlet runner bean (*P. multiflorus* Willd.).

On the lima bean the organism is quite virulent, and the disease produced is similar to that on the common bean. One symptom, however, in artificially inoculated plants was very striking and seemed to be more or less peculiar to this species. When the pathogene was introduced into the stem of the plant, the bacteria entered the leaves through the vascular bundles and there killed the veins in certain areas. These veins turned a very dark red to black and gave the leaf the appearance of having been infected by the anthracnose fungus, *Colletotrichum lindemuthianum* (Fig. 3). These diseased areas, however, flag and wilt very rapidly, which is not characteristic of the anthracnose disease.

In the inoculation experiments conducted on the scarlet runner bean the disease was much less severe.

Negative results were obtained with a number of legumes and other plants. The list is as follows: The adzuki bean (*Phaseolus angularis* (Willd.) W. F. Wight), the mung bean (*Phaseolus aureus* Roxb.), the cowpea (*Vigna sinensis* (L.) Endl.), the soy bean (*Glycine max* Merr.), the broad bean (*Vicia faba* L.), the garden pea (*Pisum sativum* L.), the flowering lupine (*Lupinus polyphyllus* Lindl. var. *albiflorus* Hort.), red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa* L.), potato (*Solanum tuberosum* L.), cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill.), and tobacco (*Nicotiana tabacum* L.).

SUMMARY

1. A severe blight and wilt of the bean in New York State has been discovered to be due to a hitherto undescribed bacterium which has been given the name *Phytomonas medicaginis* var. *phaseolicola*.

2. The symptoms of the disease are described. These are not strikingly different from the symptoms of the diseases of bean due to *Phyt. phaseoli* and *Phyt. flaccumfaciens*.

3. The organism has been isolated from diseased specimens and its pathogenicity has been proved. A description of the morphology of the pathogene together with its cultural characters is given.

4. In addition to the common bean (*Phaseolus vulgaris* L.), the scarlet runner bean (*P. multiflorus*) and the lima bean (*P. lunatus* L.) have proved susceptible.

CORNELL UNIVERSITY,
ITHACA, N. Y.

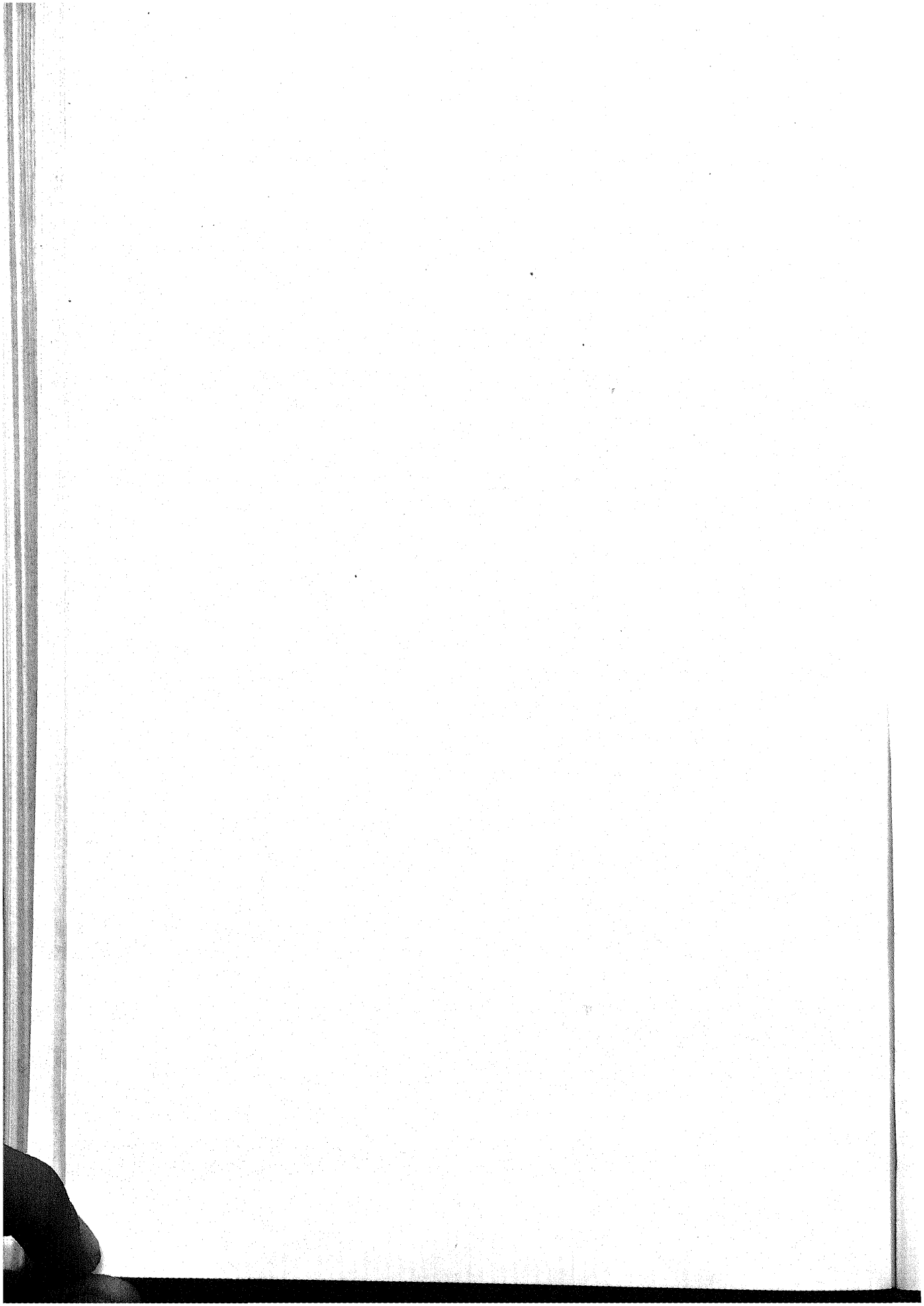
EXPLANATION OF PLATE LV

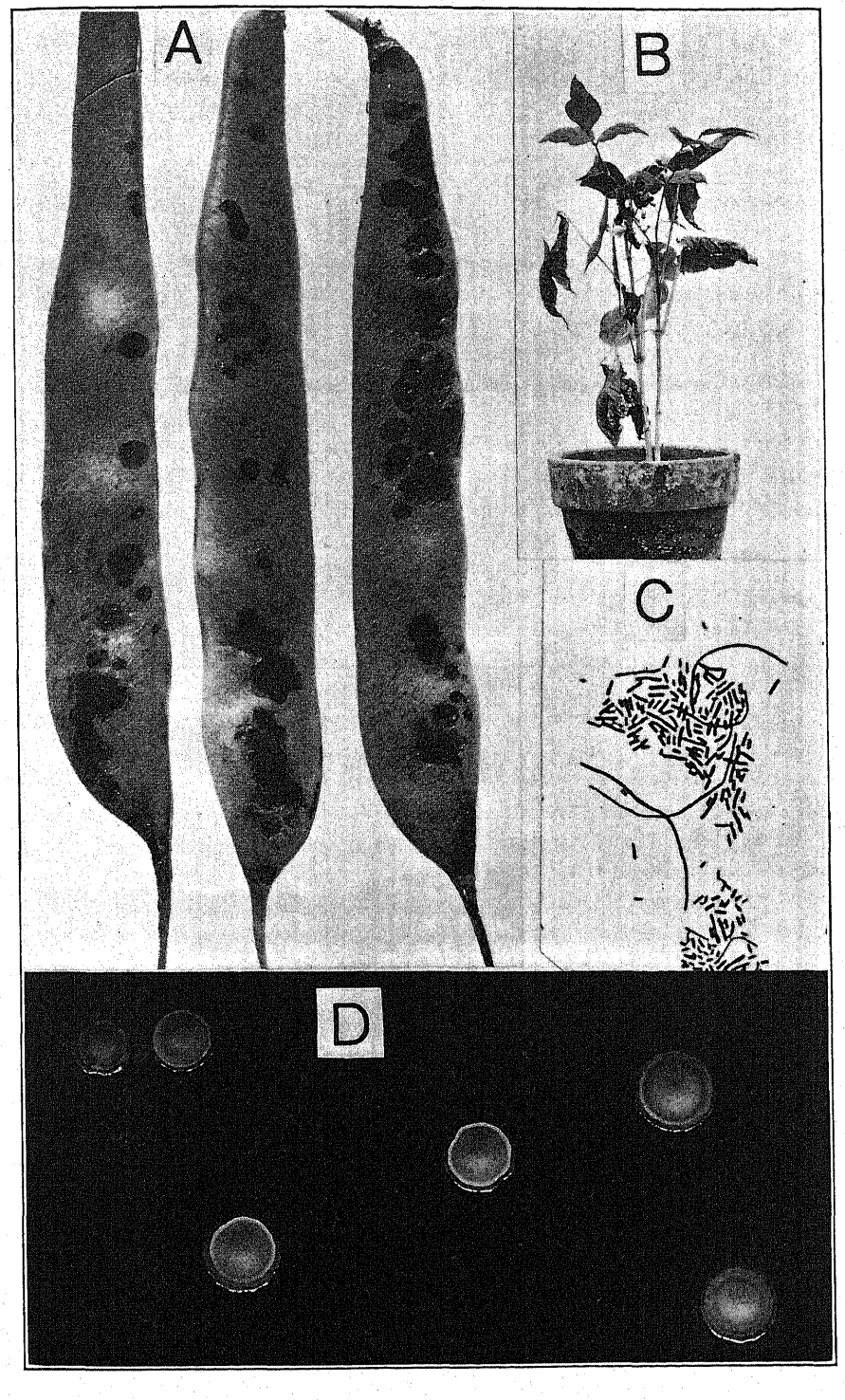
A. Pods of the Red Kidney bean showing water-soaked lesions from which *Phyt. medicaginis* var. *phaseolicola* was isolated.

B. Wilt of two Wells' Red Kidney plants due to artificial inoculation of the plants at the first leaf node with *Phyt. medicaginis* var. *phaseolicola*.

C. Photomicrograph of the bacteria from acid beef-extract agar showing filaments. Organisms stained with carbol fuchsin.

D. Colonies of *Phyt. medicaginis* var. *phaseolicola* on beef-extract agar 14 days old. 2×.




A
B
C
D

NET NECROSIS OF POTATO

D. ATANASOFF

GENERAL REMARKS

Net necrosis of potato was first described by W. A. Orton (10) in 1914 as follows: "It is characterized by the occurrence of narrow streaks or dots of browned tissues outside of the vascular ring and extending from the stem end into the tuber for a considerable distance or entirely through it. These brown tissues are free from fungi or bacteria." Orton was not sure of the cause of net necrosis.

The above short but exact description of the disease, to which Orton gave the name net necrosis, together with his illustrations, is sufficient to enable us to recognize with certainty a little understood, but not uncommon, potato disease.

C. R. Orton (9), in a short note in 1916, described the same disease. His illustration of the disease resembles the disease discussed in this paper.

Güssow (6), also in 1916, writes of this disease as follows: "During this year a considerable number of potatoes were found on being cut to have brownish internal discolorations which at first are arranged in the form of a ring, but later on become quite irregular in their distribution. These discolorations correspond to the vascular bundles . . ." Güssow observed that the disease begins at the stem end and gradually travels along the vascular bundles towards the eye-end, turning the tissues brown as it proceeds. He did not find any bacteria or fungi associated with the disease. The disease is distributed over a wide area, having been observed in New Brunswick and British Columbia. In planting tubers showing net necrosis, Güssow was surprised to find that in almost every case the disease was hereditary, *i.e.*, it was transmitted to the new tubers.

Coons (3) writes of net necrosis: "The blackened flesh is free from parasitic organisms and the disease is believed to be of some such nature as mosaic or leaf roll." This is the first mention that net necrosis is a virus disease, although Güssow's experiments suggest this. Cook (2) and Jones *et al.* (7) also state that net necrosis is inheritable from generation to generation.

Schultz and Folsom (13, 14, 15) were the first to make a study of net necrosis. They write (13) that "in Maine net necrosis has been consistently accompanied by leafroll. It may be that net necrosis, a tuber phloem-necrosis, is caused by the same virus as is leafroll or phloem-necrosis." This

hypothesis, they write, explains the available facts and explains them at least as well as does the assumption that the two diseases are caused by different viruses. According to them "net necrosis is apparently a leafroll symptom, being a discoloration which results from tuber phloem-necrosis and which appears more often as conditions of variety, recency of infection and weight of tuber are more favorable. It develops in the dormant tubers without relation to differences in the storage temperature. When it occurs as a symptom of leafroll, the effects of the latter are still more detrimental, one being a decided spindliness of the sprouts. Transmission and control of leafroll are thus concerned indirectly with net necrosis and spindling-sprout."

Gilbert (5), two years later, also came to the conclusion that "Net necrosis . . . is definitely and closely correlated with spindling-sprout of the tuber and with leafroll of the plant." This has become meanwhile the prevailing opinion among plant pathologists.

Murphy and McKay (8), on the other hand, write: "Much emphasis has been laid in America on the relationship of leaf-roll to net necrosis, that is, the presence of fine net-like strands of dead tissues in the neighborhood of the vascular ring. This feature was not found to be by any means invariably associated with leafroll."

Anderson and Millar (1), in an anonymous paper, write also: "American observers state that the tubers of plants affected with leafroll frequently show net necrosis—the presence of fine net-like strands of dead tissue in the outer part of the flesh of the tuber—associated with a spindling condition of the sprouts, but these features have not been found in Scotland to be symptomatic of leafroll."

The evidence gathered by the writer during the last three years regarding the nature of net necrosis is entirely contradictory to the results obtained by Schultz and Folsom and later also by Gilbert. It shows conclusively that net necrosis, as described and illustrated by W. A. Orton, is not a tuber symptom of leafroll, but that it is a different disease, which attacks the potato plant independently of leafroll. It is not a disease of the tuber alone but manifests itself in various ways also on the leaves of the affected plants. The leaf symptoms of this disease vary, according to variety, from what has been described by Quanjer (11, 12) as Aucuba mosaic to complete absence of Aucuba mosaic or development of Aucuba mosaic symptoms only at the beginning or at the end of the growing period.

DESCRIPTION OF THE DISEASE

Plants infected with net necrosis grow normally, are vigorous, attain normal size and show no morphologic changes whatever. Their yield is good. No apparent running out of the infected plants takes place even as far as the fourth generation after infection.

The symptoms of net necrosis vary considerably according to the variety. Some of the symptoms may be entirely absent in some varieties and very pronounced in others.

Leaf symptoms. In some varieties the leaf symptoms of net necrosis form what has been described by Quanjer (11, 12) and later by others as Aucuba mosaic of the potato plant. The affected plants and their leaves are as normal and as vigorous as healthy plants of the same variety. The leaves of such plants, however, are sprinkled or spotted with more or less rounded, bright yellow spots (Fig. 5). These spots are of considerable size, as large as one centimeter in diameter in some varieties (Midlothian Early) and very small in others. Often they coalesce, forming large, irregular,

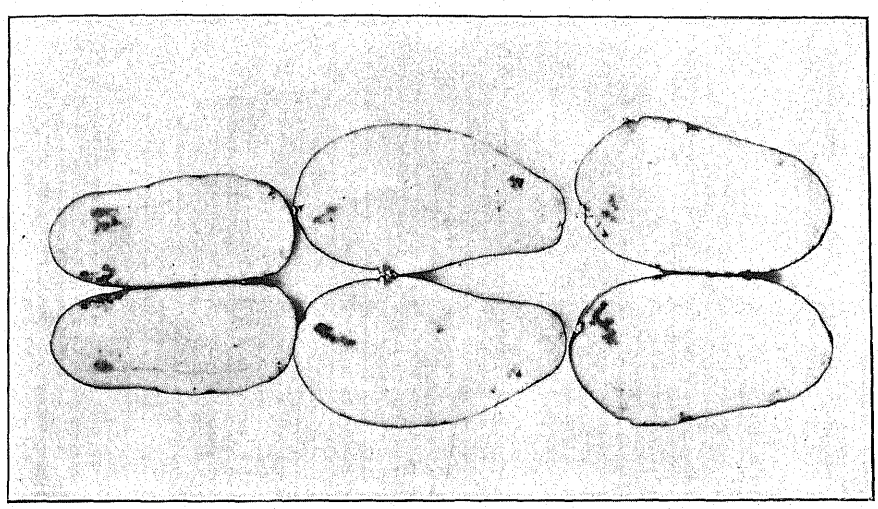


FIG. 1. Ashleaf tubers showing slight net necrosis. Rusty spots scattered irregularly all over the tubers.

yellow blotches on the leaves. When some of the older, fleshy leaves are heavily spotted, they preserve their normal shape but become blistered, *i.e.*, the yellow areas become slightly elevated above the normal green portions of the leaf. In the varieties Schotsche Muis (Midlothian Early, Duke of York), Koksiaan (May Queen), Paul Krüger (President), Ninetyfold, etc., the leaves can be so severely spotted as to resemble closely the variegation of *Aucuba japonica*—hence Quanjer's name Aucuba mosaic for this disease. In other varieties the yellow spotting of the leaves is much less pronounced and may be limited to only a few of the leaves of a plant. Such are the varieties Immune Ashleaf, Eigenheimer, Industrie, Bintje, etc. But in these varieties, also, the yellow spotting becomes more pronounced towards the end of the growing period. Other varieties, as Roode Star for instance,

show very slight yellow spotting and blistering of the lower leaves. In this case the yellow spotting resembles superficially slight frost injury. The yellow spotting of this variety, however, soon disappears or becomes less pronounced. On some of the affected leaves the yellow spots become brown and necrotic, and the browning soon extends over the top and edges of the leaf, advancing downwards until the whole leaf dies and falls off prematurely. Still other varieties, as Success, do not show any yellow spotting whatever in any stage of their development. In such cases the young plants of affected mother tubers come above the ground quite normal and absolutely free from any disease symptoms. Before the plants reach any size, however, there appear on the tips and edges of their lower leaves irregular brown to black spots as in the case of mature secondary leafroll plants. The brown

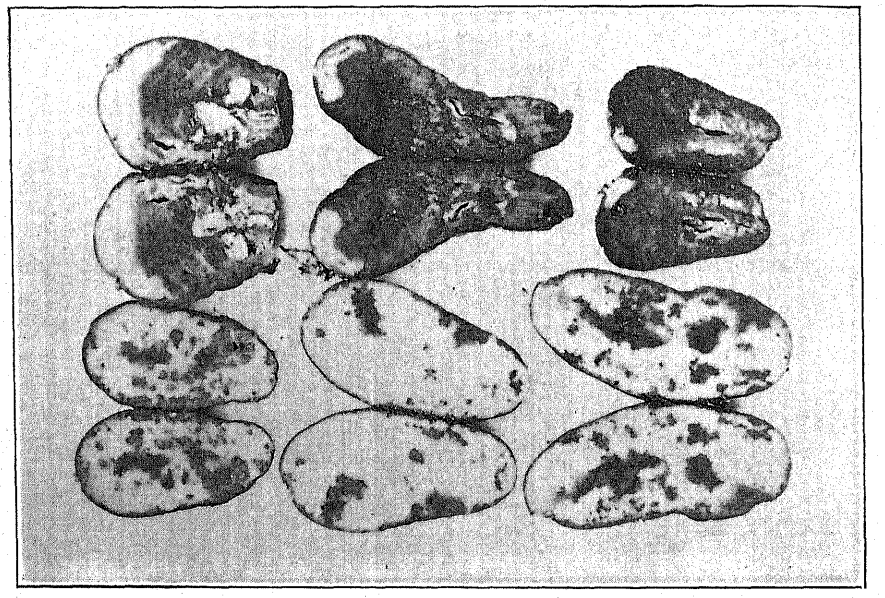


FIG. 2. Ashleaf tubers showing severe symptoms of net necrosis. Note that some tubers are wholly destroyed and are being invaded by fungi.

spots grow in area, coalesce and gradually extend over the whole upper portion of the leaves. These affected leaves thus shrivel, die, and fall off prematurely, disclosing the base of the stems. The uppermost leaves of such plants remain permanently free from any pathogenic changes. Finally there are varieties, like Bravo for instance, on which leaf symptoms have never been seen.

Of the numerous plants belonging to a large number of different varieties artificially and naturally infected with net necrosis, none ever showed any

leafroll symptoms, although all varieties used are known to be susceptible to and capable of developing pronounced symptoms of leafroll when infected with this disease.

Tuber symptoms. The tuber symptoms of net necrosis also vary according to variety. They are most pronounced and develop most rapidly in the early varieties, Schotsche Muis (Midlothian Early) and Ashleaf (Fig. 2). In most varieties the tuber symptoms are absent or only slightly developed at the time of digging. It is in the storage houses and during the winter that the tuber symptoms of net necrosis, if ever, become apparent and reach maximum development. The early varieties, however, show distinct net necrosis of the tubers at the time of lifting, which may be as early as the beginning of June. In these varieties the necrosis of the tubers increases with great rapidity, and within two months after lifting the entire sub-

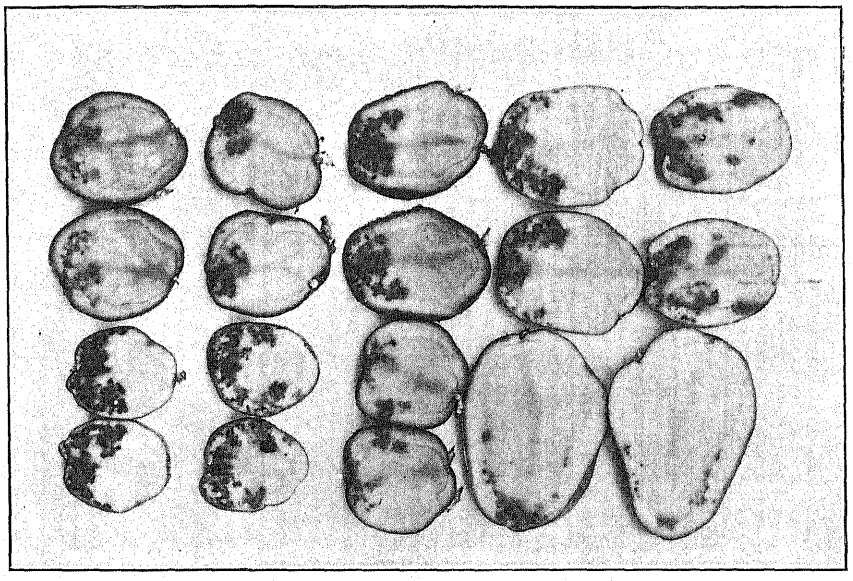


FIG. 3. Roode Star tubers showing net necrosis. Note that the brown spots first appear at the rhizome-end and advance gradually towards the eye-end of the tuber.

stance of some tubers may become wholly brown and necrotic (Fig. 2). Long before this stage the tubers have become unfit for consumption. In the varieties Midlothian Early and Ashleaf the tuber necrosis may develop very rapidly and destroy completely some of the infected tubers, while other tubers originating from the same plant may show only slight necrosis, which does not develop further. Such tubers remain alive until spring and serve for further propagation of the disease. Freshly dug tubers of the above

two varieties, when fully ripe, will show, upon careful examination, more or less pronounced rusty brown spots deep under the periderm in the flesh of the tuber. Gradually these rusty spots extend upwards against and along the periderm, forming large rusty brown, watery blotches under the periderm which later become dry. Soon the periderm dries out and begins to shrivel or sink in over the necrotic area. This condition of the diseased tuber, as well as the other symptoms, resembles very much the symptoms of Phytophthora tuber infections, and there can be no question but that the tubers infected with net necrosis have often been considered as being infected with *Phytophthora*.

Potato tubers affected with net necrosis, when cut open longitudinally, show very pronounced rusty brown blotches, spots, or streaks in the parenchyma. In some varieties (Roode Star) the first rusty spots appear in the parenchyma tissues immediately under the rhizome scar of the tuber (Fig. 3) and extend gradually inward and upward toward the eye end of the tuber. In other varieties (Midlothian Early, Ashleaf) the necrotic spots may appear in any portion of the tuber (Figs. 1 and 2), but very seldom or never at the stem end. In both cases the rusty spots appear outside of the vascular ring in the parenchyma of the tuber, as first stated by W. A.

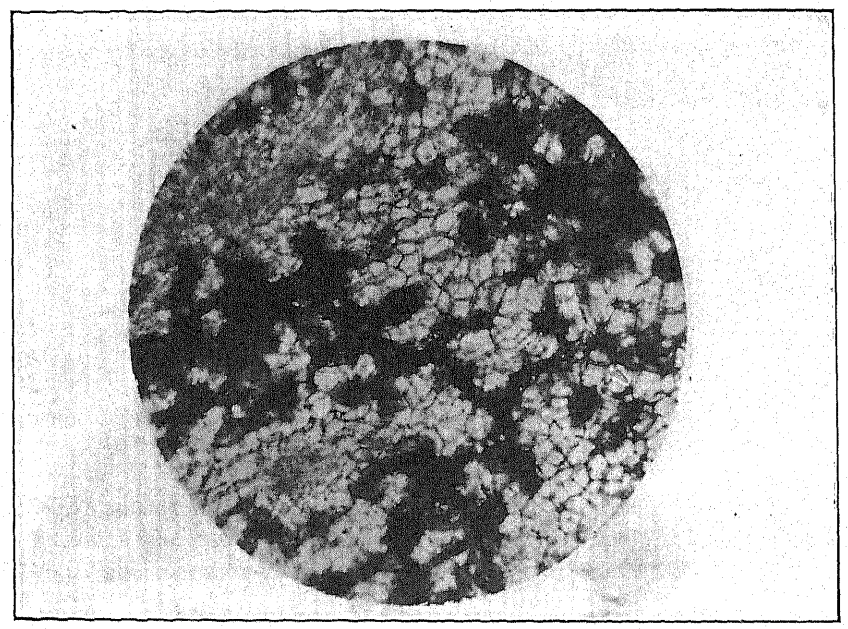


FIG. 4. Photomicrograph of a section of potato tuber of the variety Roode Star infected with net necrosis.

Orton (10), and have no relation to the phloem, as claimed by Schultz and Folsom (13). This difference in symptoms of net necrosis, as studied by Schultz and Folsom on the one hand and by Orton and the writer on the other hand, may be due to the fact that different varieties react differently to the disease. It also may be due to the fact that Schulz and Folsom's net necrosis material was infected also with leafroll. The presence of the latter in the tubers, as is known, may lead to the development of phloem necrosis in some cases. The probability that Schultz and Folsom have worked with an entirely different pathologic condition of the potato tuber than the one observed by Orton and the one discussed in this paper is very small. The illustration of net necrosis shown in Plate 2B of the paper by Schultz and Folsom (13) agrees completely with the net necrosis described and illustrated by Orton (10) and illustrated in this paper by the variety Roode Star.

Microscopic examination of the tubers before the appearance of macroscopic symptoms of the disease shows that the cell contents of individual cells or small groups of cells in the tuber parenchyma are brown colored and disorganized. The browning extends gradually to the neighboring cells and becomes visible to the unaided eye. The number of spots increases, and some of them coalesce, forming a network (Fig. 4). Such tubers, when planted, give rise to large and vigorous plants.

EXPERIMENTAL RESULTS

During the spring of 1922, after the appearance of Schultz and Folsom's paper (13) in which they claimed that net necrosis is a tuber symptom of leafroll, many hundreds of tubers from leafroll plants belonging to a large number of different varieties were cut and examined under the direction of Professor Quanjer, but in no case was net necrosis to be seen. At the same time there were at the writer's disposal several bushels of tubers of the variety Success and several hundred tubers of the variety Ashleaf originating from nonleafroll plants, which had almost 100 per cent net necrosis.

The disease present in this material closely resembled the Phytophthora tuber infection. A considerable number of the tubers were cut open and left in a moist chamber for several days, but in no case did any *Phytophthora* develop on them, as is usually the case with tubers infected with this fungus. A number of the infected tubers of both varieties were planted to see whether they would show any leafroll symptoms. In all cases the tubers gave rise to vigorous and leafroll-free plants. Since 1922 a portion of the Ashleaf material has been planted every year by the writer, and in all cases the plants have been free from leafroll; but in all seasons they showed more or less pronounced Aucuba mosaic on the leaves and net necrosis in the tubers.

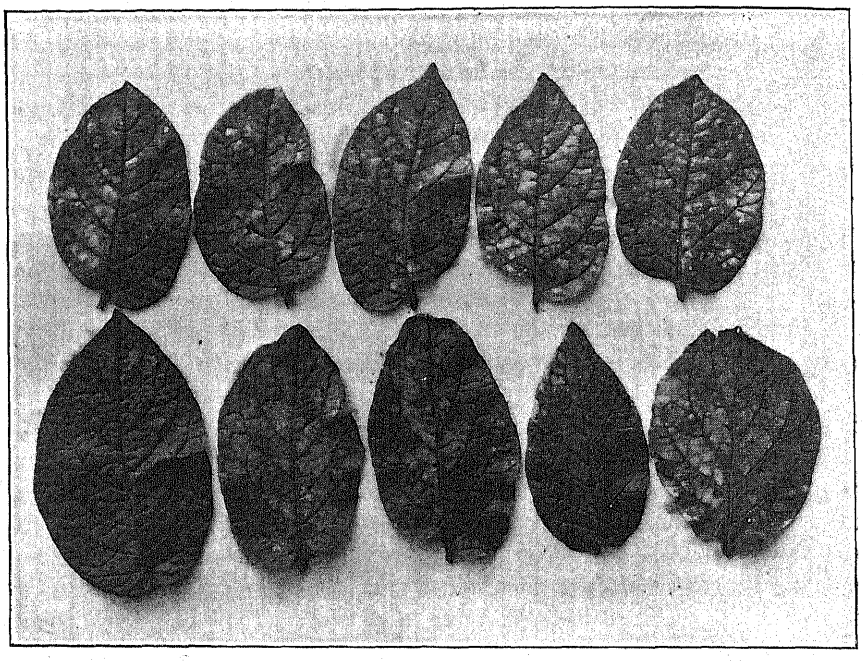


FIG. 5. Potato leaflets of the variety Midlothian Early showing Aucuba mosaic.

Early in 1924 Dorst (4) published his work on bud variation in the potato plant, in which he reported that a selection of the variety Roode Star, contrary to the general experience, developed internal brown spot on all soils. Dorst gave several tubers of this selection to Prof. Quanjer, who turned them over to the writer for further investigation. Examination showed that it was not affected with internal brown spot, but with net necrosis. When a portion of this material was grafted to healthy tubers of Midlothian Early, the necrotic condition was transmitted to the grafted healthy tubers. The rest of the material was planted on heavy clay soil, on which internal brown spot has never been seen in Holland. The resulting plants grew normally, showed no leafroll, but slight temporary symptoms of Aucuba mosaic. Their tubers developed net necrosis after lifting in all cases. While the above plants were still young and growing vigorously, their shoots were grafted on young Midlothian Early plants. The grafted plants were kept isolated in the hothouses and were fumigated regularly to keep them free from aphids. These plants did not develop distinct Aucuba mosaic symptoms. Their tubers, however, showed very severe net necrosis, while those of the check plants were free from net necrosis. The absence of Aucuba mosaic symptoms on the grafted plants is due to the fact

that the plants were not growing normally in the hothouse. Net necrosis tubers planted during the spring of 1925 in the hothouse also failed to show Aucuba mosaic in a distinct form, although the same material planted outside gave rise to plants which showed distinct Aucuba mosaic.

In the fall of 1924 the writer received from Dr. Oortwijn Botjes several hundreds of disease-free tubers of the variety Roode Star. Upon examination all tubers without exception were found to be affected with net necrosis. Dr. Oortwijn Botjes had also observed that the tubers of this lot had rusty brown spots at the rhizome end, but he, like many others, considered this due to *Phytophthora*. Although all this material was infected with net necrosis, it originated from plants that had been grown isolated from other plants for several years and which had never shown any symptoms of any of the degeneration diseases. This material, therefore, seemed especially suited for infection experiments, because it could be taken for granted, according to experience with the disease-free potato seed furnished by Oortwijn Botjes, that it was free from other virus diseases as leafroll, mosaic, etc.

The first step in the study of this, as well as of the other, net necrosis material was to establish whether the tubers showing net necrosis, give rise to leafroll plants when planted. Twenty tubers of the Roode Star material showing net necrosis were planted on light sandy soil. The resulting plants were normal and vigorous, and none of them showed any leafroll symptoms whatever. Several hundred tubers of the same lot were planted in a second field on heavy clay soil. They also gave rise to normal, and absolutely leafroll-free plants.

Ten tubers of the same material, showing net necrosis, were grafted early in February, 1925, with tubers of the variety Paul Krüger (President), originating from leafroll plants. The grafted tubers were incubated for two months at room temperature. The Paul Krüger tuber portions were then removed and the net necrosis portions planted out of doors. Seven of the ten tubers thus treated developed, within a short time, typical and very pronounced symptoms of secondary leafroll. This experiment shows conclusively that net necrosis is not a tuber symptom of leafroll.

In order to see the effect of net necrosis on different potato varieties, ten disease-free tubers of each of the varieties Bravo, Midlothian Early, Eigenheimer, Immune Ashleaf, Bintje, May Queen, Industrie, and Success were grafted early in February with Roode Star tubers affected with net necrosis. The grafted tubers were kept at room temperature for about 50 days and were planted March 27 on sandy soil well fertilized with artificial fertilizers. Ten tubers of the naturally infected Roode Star from Oortwijn Botjes were also planted with the above varieties. All tubers gave rise to normal and vigorous plants. All Roode Star plants from the naturally infected tubers showed slight Aucuba mosaic symptoms while still young. However,

gradually all Aucuba mosaic symptoms disappeared completely. Meanwhile some of the lower leaves became rusty and died. This, however, does not attract much attention and is never very distinct in this variety. Towards the end of the growing period the same plants were normal, large, and vigorous. Such plants would be considered as perfectly healthy. This explains why Dr. Oortwijn Botjes's whole stock of this variety was 100 per cent net necrosis.

The variety Bravo in this experiment showed no leaf symptoms. All ten plants were normal and free from any disease symptoms.

The variety Midlothian Early (Schotsche Muis), on the other hand, developed in all ten plants very pronounced Aucuba mosaic on the leaves, which became more and more pronounced during and towards the end of the growing period. At this time most of the riper tubers showed distinct necrosis as described for this variety. The rest of the tubers in all cases later developed net necrosis. The check plants of this variety, more than 50 in number, did not show any Aucuba mosaic, and only four per cent of their tubers developed net necrosis in storage.

All ten plants of the variety Eigenheimer showed Aucuba mosaic, but the symptoms were less pronounced and usually limited to a few leaves of each plant. Here also the plants grew normally and were very vigorous. The check plants of this variety were also free from Aucuba mosaic.

The ten Immune Ashleaf plants also showed distinct Aucuba mosaic, slight at first but later pronounced. Here, however, the yellow spots were not bright yellow, as in Midlothian Early, but more of a yellowish green tint. The check plants did not show any Aucuba mosaic.

All ten plants of the variety Bintje showed Aucuba mosaic. The yellow spotting, although quite distinct, was not pronounced at first, but became more and more so toward the end of the growing period. Here also all check plants remained healthy.

The ten May Queen (Koksiaan) plants behaved as the Bintje plants. The check plants remained free from Aucuba mosaic.

The Industrie plants at an early stage showed distinct Aucuba mosaic symptoms, consisting of numerous very small, often coalescing yellow spots, most common and numerous at the tips of the uppermost leaflets of the lower leaves. Gradually the tips of these leaves became rusty. The necrotic spots or rustiness gradually spread over the whole leaf, extending after a time to the leaves higher up on the stem. The check plants remained free from Aucuba mosaic.

The Success plants came up above the ground normal and vigorous and remained for some time absolutely free from any disease symptoms. Gradually during the growing period, there appeared on some of the lower leaves more or less numerous, small, rounded, yellow spots, followed by necrotic

spots. The tops of all ten plants remained normal and free from any disease symptoms until the end of the growing period.

The relation of Aucuba mosaic to net necrosis was apparent at a very early date. At first, however, it was thought that the appearance of Aucuba mosaic on plants from tubers affected with net necrosis might be incidental, as appeared to be the case with leafroll in Schultz and Folsom's work. The constant appearance of Aucuba symptoms on plants from many hundreds of net necrosis tubers from five different sources, and of four different varieties, made it quite improbable that the occurrence of the two diseases on the same plants is incidental.

In order to check the above results, ten healthy Midlothian Early tubers were grafted with ten tubers of the variety May Queen (Koksiaan), originating from plants showing pure Aucuba mosaic. The latter were taken from Prof. Quanjer's collection of virus disease in pure form, from the material which had served for the description of Aucuba mosaic.

The grafted tubers were kept for 50 days at room temperature and were planted on March 23 in well fertilized sandy soil. All plants developed pronounced Aucuba mosaic symptoms. During the second half of June the tubers of all the ten plants showed more or less severe, but in every case distinct, net necrosis when lifted. The neighboring row of check plants showed no Aucuba mosaic and no net necrosis in the tubers.

This experiment shows that even the supposedly pure Aucuba mosaic material used by Quanjer for the description of, and infection work with, this disease transmitted net necrosis to the healthy plants.

SUMMARY

Net necrosis is a tuber symptom, not of leafroll, but of Aucuba mosaic. Spindling sprout, supposed by some to develop on those potato tubers affected with leafroll and with net necrosis, has no relation to leafroll.

INSTITUUT VOOR PHYTOPATHOLOGIE,
WAGENINGEN, HOLLAND.

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BIOCHEMISTRY OF PLANT DISEASES VIII
ALCOHOLIC FERMENTATION OF FUSARIUM LINI[1]

HOUSTON LETCHER and J. J. WILLAMAN

INTRODUCTION

As a continuation of earlier work by Anderson (2) from this laboratory on the biochemistry of *Fusarium lini,* a brief study has been made with regard to alcohol production by the organism. The four principal objects of the present work were the following: (a) to determine the time required for maximum production of alcohol by the organism; (b) to ascertain the alcohol production on different types of saccharine media; (c) to measure the alcohol production of several cultures of the organism, and to correlate, if possible, alcohol production with pathogenicity; and (d) to identify any reaction products of the metabolism of the organism, other than alcohol and carbon dioxide.

With regard to alcohol production by *Fusarium lini,* Anderson (2) reported a value of 3.44 per cent by weight, and that approximately the same percentage of alcohol was toxic to the fungus when cultured on it. This led to the conclusion that the fungus produced alcohol to the point where the concentration became toxic and then used the alcohol so formed as a source of food. Various workers have studied the alcohol production of numerous organisms, but *Fusarium lini* is unique in producing alcohol not only from hexoses but from pentoses as well.

In the course of the present work it was found that *Fusarium lini* gave qualitative tests for acetaldehyde as one of its metabolism products. Neuberg and his associates (6, 7, 8, 9, 10), using $CaSO_3$ and Na_2SO_3 as fixatives [*cf.* Connstein and Luedecke, Ger. Pat. No. 298593/12o. 1915], showed that acetaldehyde was an intermediate compound in fermentations by bacteria and by yeasts.

EXPERIMENTAL METHODS AND MATERIALS

Source of Fusarium lini used. The cultures of *F. lini* used in the present investigation were obtained from the Division of Plant Pathology, Uni-

[1] Published with the approval of the Director as Paper No. 626, Journal Series, Minnesota Agricultural Experiment Station. The paper is abridged from a Master's thesis by the senior author in the University of Minnesota, 1925.

versity of Minnesota, through the courtesy of Dr. Louise Dosdall and Mr. W. C. Broadfoot.[2]

Preparation of media. The composition of the different media are specified in subsequent paragraphs. In general, the media were autoclaved at 10 pounds pressure for 20 minutes. One hundred cc. of liquid were used in a 300-cc. Erlenmeyer flask.

Inoculation. The experimental cultures were inoculated by using small bits of mycelium from well developed stock cultures on liquid media.

Incubation. Culture flasks were kept in incubators at a temperature of 26° to 28° C.

Culture media. Sucrose, 8 and 12 per cent, was used in the following salt-peptone solution:

Monopotassium phosphate	1.00 gm.
Magnesium sulfate	0.25
Ferrous sulfate	0.10
Peptone	7.50
Water to make one liter.	

Maltose syrup.—The original syrup of Brix reading 60 was diluted until a reading of 7.6 was obtained.

Corn stalk syrup.—This material was prepared at the Minnesota Experiment Station in 1921. The syrup was diluted to a Brix reading of 11.2.

Beerwort.—This material was furnished through the courtesy of the Minneapolis Brewing Company. Both the original wort of Brix reading 18.45 and diluted portions of Brix 8.0 were used.

Glucose, 8 and 12 per cent, was used in the following salt medium:

KH_2PO_4	0.50 gm.
$MgSO_4$	0.25
NH_4NO_3	1.00
Water to make one liter.	

The glucose used was the commercial "Cerelose."

Molasses.—A commercial brand of light New Orleans molasses was diluted to the desired densities.

"Black liquor" from paper mill.—This material was from the Northwest Paper Company, Cloquet, Minnesota. It was classified as "Keebra black liquor from tamarack cook" and had a Brix reading of 34.2. A portion of the liquor dialyzed in a hardened collodion bag for a week was also used.

[2] Broadfoot's results on the cultural characters and pathogenicity of these cultures are given in a separate article in this number of PHYTOPATHOLOGY (Phytopath. 16: 951–978. 1926).

Alcohol determination. One hundred cc. of culture medium was filtered through cheesecloth to remove the mycelium, and the felt was then pressed as dry as possible to remove adhering liquid. Fifty cc. of distilled water was added to the filtrate and the mixture placed in a 1,000-cc. flask connected to a straight tube condenser. The first 100 cc. of distillate were collected and the alcohol in it determined by the specific gravity method, using a chainomatic specific gravity balance.

Qualitative and quantitative tests. The following tests were used on the distillates to determine acetaldehyde, acetone, and alcohol:

Acetaldehyde—Tollen's reaction, according to Mulliken (5).
Schiff's test, according to Weston (12).
The semi-carbazone reaction, as given by Weston (12).
Beta-naphthol test, according to Mulliken (5).
NaOH test, according to Mulliken (5).
Fehling's reaction, according to Allen (1).
Bisulfite precipitation method, originally devised by Ripper and listed by Kingscott and Knight (4).
Piperidine test, according to Neuberg (6).
Acetone—According to method of Bülow (3).
Alcohol—Iodoform, according to the method of Allen (1).

EXPERIMENTAL DATA

Alcohol Production by Two Cultures of Fusarium lini

Forms 7 and 2 were grown on the media shown in tables 1 and 2. The flasks of this series were removed from the incubators at the end of 5, 10, 15, 20, 30, and 40 days and the alcohol determinations made in duplicate. The data are presented in tables 1 and 2 and in figures 1 and 2. It is

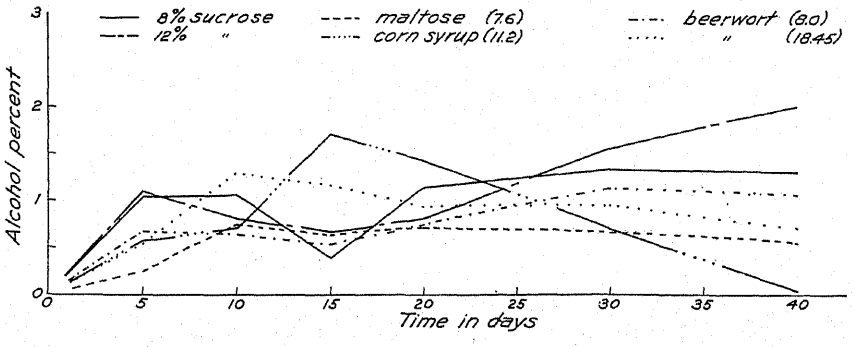

FIG. 1. Production of ethyl alcohol by form 7 of *Fusarium lini* on various media.

TABLE 1.—*Alcohol production by Fusarium lini, form 7*

Media	Age of cultures in days					
	5	10	15	20	30	40
	Alcohol production in per cent					
Sucrose (8 per cent)	1.03	1.07	0.40	1.13	1.33	1.30[a]
Sucrose (12 per cent)	1.09	1.31	0.67	0.80	1.58	2.02
Maltose (Brix 7.6)	0.23	0.73	0.43	0.70	0.67	0.53
Corn syrup (Brix 11.2)	0.57	0.70	1.71	1.44	0.70	0.04
Beerwort (Brix 8.0)	0.67	0.63	0.53	0.73	1.13	1.06
Beerwort (Brix 18.45)	0.53	1.30	1.18	0.93	0.97	0.77

[a] Alcohol determination after 45 days.

apparent that both cultures produce appreciable amounts of ethyl alcohol on all the media; that a maximum is not attained before 20 days or more; that this maximum is usually followed by a decrease; and that form 2 produces more alcohol than form 7. After 10 to 20 days practically all of the cultures had an alcoholic odor, and in the case of the corn stalk syrup cultures the odor was very similar to that of a cider fermentation. The growth of the mycelium was much the same for all the cultures after 20 days, since by that time the mycelium had covered practically all of the available liquid surface and from then on there was an increase in depth of the felts. The good yield of alcohol by form 2 on corn stalk syrup suggested the use of this culture on the syrup in an effort to increase the alcohol and to furnish material for identification trials.

TABLE 2.—*Alcohol production by Fusarium lini, form 2*

Media	Age of cultures in days					
	5	10	15	20	30	40
	Alcohol production in per cent					
Sucrose (8 per cent)	0.57	0.90	0.00[a]	1.72	1.13	1.00
Sucrose (12 per cent)	0.53	1.51	2.50	3.27	3.24[b]	2.05
Maltose (Brix 7.6)	0.50	1.68	0.97	1.44	0.66	1.19
Corn syrup (Brix 11.2)	0.77	3.17	3.52	3.34	2.22	2.19
Beerwort (Brix 8.0)	0.46	1.10	1.33	0.67	1.40	1.03
Beerwort (Brix 18.45)	0.63	1.23	1.95	1.47	2.68	2.61
Glucose (8 per cent)	0.46	1.23	2.40	2.47	1.07	0.80
Glucose (12 per cent)	0.50	0.93	1.95	2.64	3.31	2.99

[a] No growth of mycelium.

[b] Alcohol determination after 25 days.

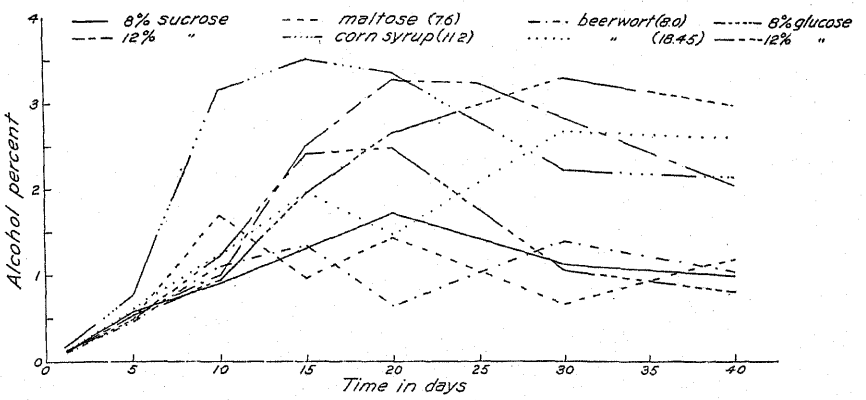

FIG. 2. Production of ethyl alcohol by form 2 of *Fusarium lini* on various media.

Alcohol Production by Nine Cultures of Fusarium lini on Molasses

To determine the differences in alcohol production among several cultures of *Fusarium lini* on a single medium, 108 flasks were prepared, using molasses of Brix reading 11.8 to 12.1. The flasks were incubated from 5 to 30 days, and every fifth day two samples in each series were removed from the incubators for alcohol determinations. The results are given in table 3 and in figure 3.

Forms 6 and 9 form a group with a relatively low alcohol production, while the others yielded high percentages of alcohol. Table 3 gives data

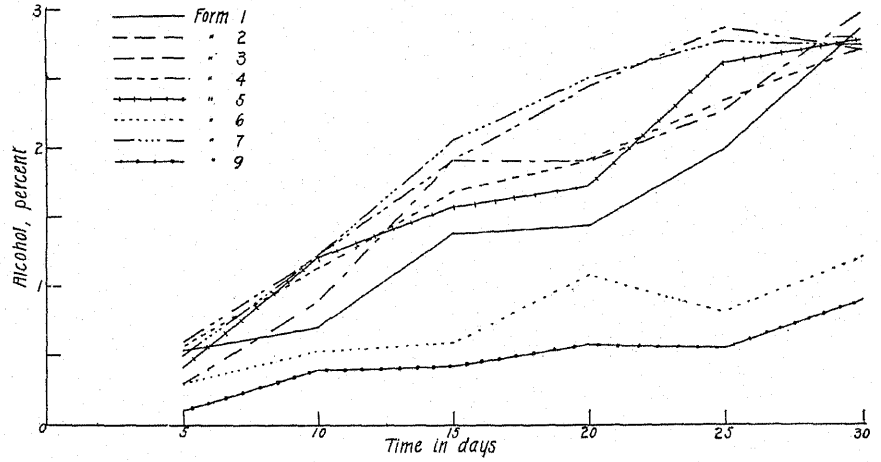

FIG. 3. Production of ethyl alcohol by various forms of *Fusarium lini* on a molasses medium.

TABLE 3.—*Alcohol production by forms of Fusarium lini on molasses*

Form used	Rank in virulence on host	Rank in alcohol production at 20 days	Age of cultures in days					
			5	10	15	20	25	30
			Alcohol production in per cent					
1	1	6	0.53	0.70	1.37	1.44	1.98	2.85
2	2	3	0.57	1.13	1.68	1.92	2.33	2.71
3	3	4	0.30	0.90	1.92	1.92	2.29	2.99
4	4	2	0.70	1.23	1.92	2.43	2.85	2.71
5	5	5	0.43	1.20	1.57	1.72	2.61	2.78
6	6	7	0.30	0.53	0.60	1.17	0.83	1.24
7	7	1	0.50	1.23	2.06	2.50	2.77	2.75
9	8	8	0.23	0.40	0.43	0.59	0.57	0.90

relative to the virulence of cultures of the fungus on flax. From these data it is evident that forms 6 and 9, with the lowest alcohol production, are also the least virulent.

Identification of End Products

To obtain larger quantities of fermentation products and to use this material for identification work, the following series of flasks were prepared:

Series A	15	flasks	10	days incubation
B	18	do	20	do
C	17	do	30	do

These flasks were kept in a room the temperature of which ranged from 20° to 31° C., but which approximated 26° to 28° C. the greater part of the time.

At the end of the growth period 500 cc. of filtrate from Series A was fractionated in a flask with a 14-inch fractionating column. One 50-cc. portion and three 100-cc. portions were obtained. Three such 500-cc. portions were fractionated and the following data obtained:

Fraction of distillate	Alcohol percentages		
	I	II	III
50 cc.	25.03	25.31	13.81
100 cc.	0.00	0.00	0.80
100 cc.	0.00	0.00	0.00
100 cc.	0.00	0.00	0.00

The 100-cc. portions were run to determine if it were possible to obtain fractions of specific gravity greater than 1.0000. A blank determination on the corn stalk syrup which had been autoclaved but not inoculated gave three 100-cc. portions of specific gravity 1.0000. The three 50-cc. portions

were combined and fractionated. The first 45 cc. of distillate had an alcohol content of 61.08 per cent, coming off largely at 78°–97° C. The second 45-cc. portion distilled at 97°–98.2° C. but principally at 98° C., and had an alcohol percentage of 1.13. The first fraction had the odor of a yeast fermentation, and was acid to litmus. It gave the iodoform test on heating, as well as in the cold, indicating the possibility of aldehyde. The latter was further shown by a positive fuchsin test.

The filtrate from the 18 flasks in Series B, 20 days old, was fractionated as in Series A. The first 50-cc. fractions (from 500 cc. of filtrate) were combined as above and distilled. The first 50 cc. boiled at 78°–79.2° C. and had an alcohol percentage of 89.0; the second 50 cc. contained 25.70 per cent alcohol; and the third, 0.00. On applying analytical tests for aldehyde to the first of these the following results were obtained:

Fuchsin aldehyde reagent	—	positive for aldehyde
Tollen's reaction	—	do
Fehling's reaction	—	doubtful for acetaldehyde
Beta naphthol test	—	negative do
NaOH test	—	positive do
Bisulphite titration	—	do do

As to the Fehling's reaction, a precipitate of copper was obtained both in the blank and in the test solutions, and the difference in quantity of the precipitates was not great enough to justify the test as positive for acetaldehyde. Mulliken (5) states that the beta naphthol test is not applicable to small amounts of the aldehyde, and the evidence obtained here was that there was but a very small amount of the acetaldehyde present in the distillates. The bisulfite titration showed 0.0064 gm. of acetaldehyde per 100 cc. of distillate.

In Series C the first fraction had an alcohol percentage of 77.75 and the bisulfite titration showed 0.0457 grams of acetaldehyde per 100 cc. of distillate.

Further Studies on End Products of Fermentation

Because of the volatility of acetaldehyde, the following distillations were carried out with the precautions suggested by Neuberg, Hirsch and Reinfurth (10). The fractionating flask was connected to a straight tube Liebig condenser and this joined by an adapter to a vertically placed coil condenser; the latter was connected by an adapter to two suction flasks connected in series, with the second flask as a safety. The flasks were immersed in an ice-bath. Thus every effort was made to obtain complete condensation of the distillates. Fifty cc. of distillate, fractionated from the filtrate from 30 culture flasks, gave an alcohol percentage of 89.05. Since

earlier tests had shown the possibility of acetone or acetaldehyde as a fermentation product, these substances were tested for in this 50-cc. fraction, as follows:

Acetone test—negative
Piperidine test—positive for acetaldehyde
Semi-carbazone reaction—positive for acetaldehyde

Tollen's reaction, NaOH test, and the fuchsin aldehyde reagent were all positive for acetaldehyde, as in the earlier tests. The bisulfite titration showed a value of 0.0512 gm. of acetaldehyde per 100 cc. of distillate.

The foregoing qualitative tests are of interest in view of the statement of Stepp (11) that the Tollen's reaction is positive for 1 part of acetaldehyde in 100,000. Furthermore, Stepp mentioned that the fuchsin aldehyde reagent is not very sensitive and that acetone did not give a color with it. Hence the facts that a good color was obtained with the fuchsin aldehyde reagent, that acetone does not give any reaction with the piperidine reagent and that the acetone test of Bülow was negative indicated the probable absence of acetone as a fermentation product. Since Stepp mentioned that the piperdine reaction was not given by formaldehyde or benzaldehyde, the latter were probably not present to interfere with the bisulfite titration. From the foregoing tests it was concluded that acetaldehyde is probably one of the fermentation products of *Fusarium lini*. The use of $CaSO_3$ as a fixative agent, as devised by Neuberg, should establish definitely whether or not acetaldehyde is an intermediate fermentation product.

Growth of Cultures of Fusarium lini on Paper Mill Waste Liquor

Three 100-cc. portions of the waste liquor from a paper mill were inoculated with forms 1 and 2, and incubated for 35 days at 26°–28° C. The liquor had a Brix reading of 34.2, but there was no growth of the fungus after 35 days. This trial was repeated with forms 2, 3, 6 and 7, but no growth resulted after 15 days incubation. Twenty cc. portions of the liquor which had been dialyzed were inoculated with forms 2, 3 and 7. Some mycelium developed after a 12-day incubation period. This result shows further the omnivorous nature of this fungus.

SUMMARY

1. *Fusarium lini,* the fungus causing flax wilt, has been cultured on six different saccharine media, and produced ethyl alcohol on all of them. This confirms the earlier observations of Anderson (2).

2. In general the maximum alcohol content of the liquid cultures was attained in approximately 30 days.

3. The alcohol production by eight forms of the fungus ranged from 0.80 to 2.99 per cent by volume 30 days after inoculation. The highest percentage for any single culture was 3.7.

4. The two forms of the organism which were the least virulent in their action on the flax plant had the lowest alcohol production.

5. Acetaldehyde apparently is one of the intermediate products formed by the fungus in fermenting sugars.

6. The acetaldehyde so derived was present at any given time only in traces, as shown by color and titrimetric determinations.

DIVISION OF BIOCHEMISTRY,
UNIVERSITY FARM,
ST. PAUL, MINN.

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STUDIES ON THE PARASITISM OF FUSARIUM LINI BOLLEY[1]

W. C. Broadfoot

The development and distribution of resistant varieties of flax has aided materially in the solution of the flax wilt problem. However, resistant varieties are not always resistant. They may be resistant in one locality and not in another. This difference in resistance may be due partly to environmental conditions, as Tisdale (10) and Barker (4) have shown that wilt resistance is only relative and may be modified by environmental conditions. It has been demonstrated that the effect of environment may be overcome to some extent by early planting (4). However, the results given in a former abstract (6) and in this paper indicate that the differences in reaction of resistant varieties in different localities may be due also to physiologic specialization of the pathogene. If this is true, it will be necessary either to select resistant varieties for certain localities, or varieties resistant to all physiologic forms.

Since Tisdale (11), Barker (4), and Anderson (1) have reviewed the literature on flax wilt, it will be unnecessary to review it again in this paper.

Five varieties of flax, namely, N. D.[2] 40013 (C. I. 241); N. D. 3080 (C. I.

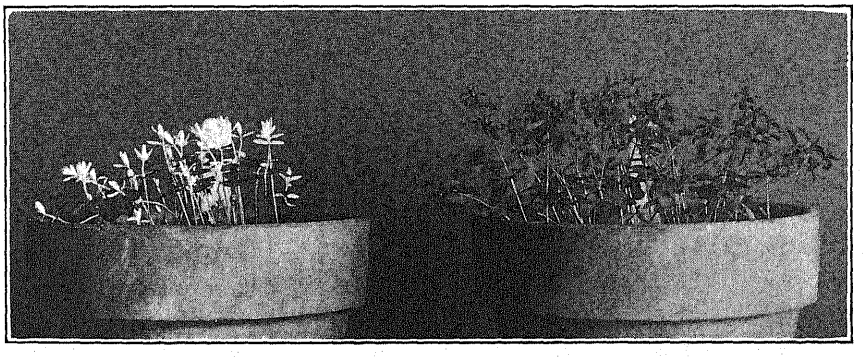

Fig. 1. Reaction to *Fusarium lini*, form 1, on Primost, Minn. 25 (C. I. 177), in pots. Check pot on the right.

[1] Published with the approval of the Director as paper No. 657 of the Journal Series of the Minnesota Agricultural Experiment Station.

The writer desires to acknowledge his indebtedness to Dr. E. C. Stakman for his helpful advice and criticism.

[2] N. D. = North Dakota Agricultural Experiment Station accession number.

C. I. = Office of Cereal Investigations accession number.

Minn. = Minnesota Agricultural Experiment Station accession number.

275); Slope (C. I. 274); Winona, Minn. 182 (C. I. 179); and Chippewa, Minn. 181 (C. I. 178), when grown at Mandan, N. D., were highly resistant to flax wilt and yielded well, according to data of Stoa and Dillman (9). When grown at University Farm, St. Paul, Minn.,[3] on the other hand, the first three varieties were more or less susceptible to wilt and yielded less than the other two, which were highly resistant also at University Farm. Table 1 shows the striking differences in yields when the varieties were

TABLE 1.—*Yields in bushels per acre of flax varieties grown at Mandan, North Dakota, and University Farm, St. Paul, Minnesota*

Flax varieties	C.I.[a]	Yields in bushels per acre	
		Mandan, N. Dak.	Univ. Farm, St. Paul, Minn.
N. D. 40013	241	11.5	1.89
N. D. 3080	275	11.3	1.31
Slope	274	10.1	3.19
Winona, Minn. 182	179	10.4	9.01
Chippewa, Minn. 181	178	10.2	10.20

[a] C. I. = Office of Cereal Investigations accession number.

grown at University Farm, St. Paul, Minn., and the lack of significant differences at Mandan, N. D.

Inasmuch as there seemed to be strong circumstantial evidence of physiologic specialization in *Fusarium lini,* the writers attempted to ascertain whether the fungus actually was specialized, and to determine the number of forms, their morphologic characteristics, parasitic capabilities, degree of stability, and mode of action.

MATERIALS AND METHODS

Table 2 lists the sources of material for cultures of *F. lini.* Four varieties of flax (*Linum usitatissimum* L.), namely, Primost, Minn. 25 (C. I. 177); Winona, Minn. 182 (C. I. 179); N. D. 3080 (C. I. 275); and N. D. 40013 (C. I. 241) were used as differential hosts for inoculation experiments. The varieties were supplied by Dr. A. W. Henry, of the Division of Plant Pathology, University of Minnesota. Cultures, accession Nos. 1, 2, 4, to 17, inclusive, are of monosporous origin. These were obtained by picking up single germinating spores with a sterile platinum needle from poured agar plates. In the case of culture No. 3, a single chlamydospore-like body in a hyphal thread was picked up. Stock cultures were grown on potato dextrose agar and were kept at room temperature.

[3] Unpublished results furnished by Doctor A. W. Henry, University Farm, St. Paul, Minnesota.

TABLE 2.—*Source of materials for cultures of Fusarium lini isolated from flax*

Accession no.	Locality or origin	Transferred from	Collector or sender	Isolated by	Date isolated or transferred
1	Agronomy plot, St. Paul, Minn.		W. C. Broadfoot	W. C. Broadfoot	Aug. 2, 1924
2	Austin, Minn.		J. J. Christensen	W. C. Broadfoot	July 24, 1924
3	Cadillac, Sask.		R. G. Olgivie	W. C. Broadfoot	Nov. 6, 1924
4	Cadillac, Sask.		R. G. Olgivie	W. C. Broadfoot	Nov. 6, 1924
5	Crookston, Minn.		A. W. Henry	W. C. Broadfoot	Sept. 2, 1924
6	Crookston, Minn.		A. W. Henry	W. C. Broadfoot	Sept. 2, 1924
7	Fargo, N. D.	Minn. Herb. culture no. 53	H. D. Barker	H. D. Barker July 5, 1921	Aug. 30, 1924
8	Kenyon, Minn.	Minn. Herb. culture no. 52	H. D. Barker	H. D. Barker March 11, 1921	Aug. 30, 1924
9	Morris, Minn.		J. J. Christensen	W. C. Broadfoot	July 24, 1924
10	Plant Pathology plot, St. Paul, Minn.		W. C. Broadfoot	W. C. Broadfoot	Aug. 2, 1924
11	Red Lake Falls, Minn.		A. W. Henry	W. C. Broadfoot	Aug. 20, 1924
12	Saskatoon, Sask.		G. Boyd	W. C. Broadfoot	Nov. 6, 1924
13	St. Lawrence, Minn.		A. W. Henry	W. C. Broadfoot	Aug. 2, 1924
14	Winnipeg, Man.		G. R. Bisby	W. C. Broadfoot	Sept. 30, 1924
15	Winnipeg, Man.		G. R. Bisby	W. C. Broadfoot	Sept. 30, 1924
16	St. Paul, Minn.	Minn. Herb. culture no. 58	H. D. Barker	H. D. Barker May 20, 1921	Sept. 30, 1924
17	do	Minn. Herb. culture no. 60	H. D. Barker	H. D. Barker Nov. 25, 1921	Sept. 30, 1924
19	do	Minn. Herb. culture no. 95	O. S. Aamodt	O. S. Aamodt July, 1915	Mar. 6, 1925
20	do	Minn. Herb. culture no. 16	H. D. Barker	H. D. Barker 1919	Mar. 6, 1925

TABLE 3.—*Results of inoculating four varieties of flax with nine physiologic forms of Fusarium lini*

Physiologic form	Accession no.	Trial	Varieties and Reactions[a]													
			Primost, Minn. 25				Winona, Minn. 182				N. D. 3080				N. D. 40013			
			Surviving plants			Reaction	Surviving plants			Reaction	Surviving plants			Reaction	Surviving plants			Reaction
			Total no.	Percent	Pct. of two trials		Total no.	Percent	Pct. of two trials		Total no.	Percent	Pct. of two trials		Total no.	Percent	Pct. of two trials	
1	3	1	0	0.0		C	0	0.0		C	3	3.7		C	1	1.0		C
		2	0	0.0	0.0	C	0	0.0	0.0	C	0	0.0	1.9	C	0	0.0	0.5	C
2	7	1	2	2.0		S	7	10.9		S	8	10.0		S	9	9.8		S
		2	0	0.0	1.0	S	1	1.8	6.4	S	0	0.0	5.0	S	4	7.2	8.5	S
3	9	1	0	0.0		S	4	6.2		S	16	20.0		R	0	0.0		S
		2	2	3.8	1.9	S	9	17.9	11.5	S	16	27.5	23.5	R	10	12.4	6.2	S
4	8	1	6	5.7		S	23	36.6		R	5	6.3		S	17	18.8		S
		2	12	16.3	11.0	S	37	73.6	55.1	R	6	10.3	8.2	S	10	18.3	18.5	S
5	2	1	7	6.6		S	1	1.4		S	10	12.2		S	28	30.3		R
		2	4	7.1	6.8	S	2	4.2	2.8	S	2	3.9	7.9	S	19	35.0	32.6	R
6	6	1	11	10.5		S	15	23.8		R	3	3.7		S	38	41.8		R
		2	5	9.3	9.9	S	23	46.0	34.9	R	8	13.5	8.6	S	18	33.6	37.7	R
7	4	1	6	5.7		S	18	28.6		R	26	32.2		R	11	12.2		S
		2	9	16.4	11.0	S	26	52.1	40.4	R	22	37.8	35.0	R	5	9.4	10.8	S
8	5	1	25	23.7		R	39	61.9		R	11	13.5		S	11	13.5		S
		2	25	45.3	34.5	R	24	47.9	59.9	R	0	0.0	6.8	S	0	0.0	6.8	S
9	1	1	54	56.2		R	33	52.4		R	67	76.6		R	21	23.1		R
		2	19	34.4	45.3	R	12	23.9	38.1	R	19	32.6	54.6	R	30	55.5	39.3	R
Uninoculated check		1	106	100.0			61	100.0			81	100.0			91	100.0		
		2	55	100.0	100.0		50	100.0	100.0		58	100.0	100.0		54	100.0	100.0	

[a] R = Resistant; S = Susceptible; C = Susceptible and chlorotic above cotyledons.

The inoculum for soil inoculations was produced on a sterilized medium consisting of a mixture of sterilized wheat, oats, and barley seeds combined with an equal volume of water. An equal amount of inoculum of each culture was mixed with sterilized soil in twelve 4-inch pots. Seeds of differential hosts were surface disinfected by immersion for 10 minutes in a 1-1000 $HgCl_2$ solution, then washed in sterile distilled water and allowed to dry. In each pot in experiment 1, 50 seeds of one variety were sown, and 25 seeds per pot in experiment 2. The control pots were treated in the same way, except that sterilized uninoculated medium was mixed with the soil. Two inoculation experiments, each in triplicate, were made in the greenhouse, one in March and the other in June. The number of plants surviving in the control pots was considered as 100 per cent. Varieties were arbitrarily designated resistant if more than 20 per cent of the plants survived.

RESULTS

Various degrees of resistance and susceptibility appeared. On this basis nine physiologic forms of *F. lini* were recognized. The results of the inoculations with nine forms are summarized in table 3.

An examination of table 3 shows that forms 1 and 2 are somewhat similar in pathogenicity. However, form 1 causes the plants to lose their chlorophyll, a condition which is brought about by no other form (Fig. 1). When the plants are from 4 to 6 inches high the green color gradually disappears from all the tissues above the cotyledons until this portion of the plant eventually becomes entirely colorless. The line of demarcation is very sharp between the normal and "chlorophyll deficient" tissues (Fig. 2). Sections of these tissues show that the chloroplasts are decidedly deformed as well as colorless. The affected plant eventually dies. This condition was produced by artificial inoculation in the field as well as in the greenhouse. Form 1 also caused a similar condition on Manchuria barley, Victory oats, and Rosen rye. The green color gradually disappeared from all the tissues above the first leaf.

The forms are numbered in order of their virulence. All four varieties are susceptible to forms 1 and 2, which are the most virulent. Only one variety, a different one for each of the forms, is resistant to forms 3, 4, and 5. Similarly, two varieties are resistant to forms 6, 7, and 8. Form 9 is the least virulent. All four varieties are resistant to this form, although it kills some of the plants of all varieties. For convenience in distinguishing physiologic forms of *F. lini*, an analytical key of the simple dichotomous type is presented in table 4.

Table 5 gives the place of collection of the nine forms of *F. lini* used in the experiments. No attempt was made to determine the prevalence and

TABLE 4.—*Analytical key to physiologic forms of Fusarium lini*

Chlorosis absent		
Primost, Minn. 25 (C. I. 177)	Resistant	
N. D. 3080 (C. I. 275)	Resistant	Form 9
N. D. 3080	Susceptible	Form 8
Primost	Susceptible	
N. D. 3080	Resistant	
Winona, Minn. 182 (C. I. 179)	Resistant	Form 7
Winona	Susceptible	Form 3
N. D. 3080	Susceptible	
N. D. 40013 (C. I. 241)	Resistant	
Winona	Resistant	Form 6
Winona	Susceptible	Form 5
N. D. 40013	Susceptible	
Winona	Resistant	Form 4
Winona	Susceptible	Form 2
Chlorosis present		Form 1

distribution of the different forms. However, certain of the forms studied appeared to be more widely distributed than others. Thus forms 2, 3, and 7 were found both in the United States and Canada, while form 1 was found only in Saskatchewan and the other forms in Minnesota alone. But it must be borne in mind that only very few collections were made, and further studies might show the situation to be entirely different.

TABLE 5.—*Place of collection of nine physiologic forms of Fusarium lini*

Accession no.	Physiologic form	Place collected
3	1	Cadillac, Saskatchewan
7	2	Fargo, North Dakota
12		Saskatoon, Saskatchewan
13		St. Lawrence, Minnesota
20		University Farm, St. Paul, Minnesota
9	3	Morris, Minnesota
14		Winnipeg, Manitoba
15		Winnipeg, Manitoba
8	4	Kenyon, Minnesota
2	5	Austin, Minnesota
19		University Farm, St Paul, Minnesota
6	6	Crookston, Minnesota
11		Red Lake Falls, Minnesota
4	7	Cadillac, Saskatchewan
17		University Farm, St. Paul, Minnesota
5	8	Crookston, Minnesota
1	9	Agronomy plot, University Farm, St. Paul, Minnesota
10		Plant Pathology plot, University Farm, St. Paul, Minnesota

MORPHOLOGY OF THE PHYSIOLOGIC FORMS OF FUSARIUM LINI

The results of these experiments indicate clearly that there are physiologic forms of *F. lini*. Experiments were then made to determine whether there are differences in morphology as well as in pathogenicity. The nine forms of *F. lini* were grown on potato plugs, steamed rice, hard oat agar, sweet clover stems, alfalfa stems, 2 per cent potato dextrose agar, and 5 per cent potato dextrose agar, all in test tubes, according to the formulae recommended by Wollenweber, Sherbakoff, Reinking, Johann, and Bailey (12) for the identification of species of *Fusarium*. *F. lini* was first described by Bolley (5) from cultures grown on slightly acid peptone agar. Such an agar was made, consisting of 2 grams of peptone, 2 grams of sugar, 2 grams of agar, a few drops of lactic acid, and 1,000 cc. of water.

Spore measurements were made with an eye-piece micrometer. A 10 per cent aqueous solution of glycerine was used for mounting the spores on the glass slides.

Comparisons of mean length and width of different magnitude of spore populations of Fusarium lini, form 1, grown in the same medium

Separate spore populations of 50, 100, and 200 spores of *F. lini*, form 1, grown on 5 per cent potato dextrose agar 56 days old, were measured for length and width. From the data thus obtained, biometric constants were calculated by the assumed mean method (3). They are given in table 6. The differences in mean length and width of each spore population were then compared in relation to their probable errors (table 7). The differences between lengths of 50 spore and those of 100 and 200 spore populations are more than three times the probable error of the difference. When the mean length of 100 spores is compared with that of 200, the difference in the two means is less than one times the probable error of the difference. It would seem, therefore, that 50 spores may not constitute a representative random sample for drawing conclusions, but that 100 spores will probably be sufficient for a comparison of the spore lengths of *F. lini*. Similarly, it appears that measurements of 50 spores might be sufficient to determine spore width. However, in all cases of further comparative spore measurements, 100 spores were individually measured for length and width.

Comparisons of the length and width of 100 spores of Fusarium lini, form 1, grown on different media

Measurements were made of 100 spores of form 1 grown on 3 different media and are listed in table 8. In one case the spores were taken from a culture of form 1, 14 days old, grown on slightly acid peptone agar. The cultures grown on sweet clover stems and on 5 per cent dextrose agar were 56 days old. The mean length of the spores from the 5 per cent potato dex-

TABLE 6.—*Variations and constants for length and width of spores of Fusarium lini, form 1, based on measuring populations of different magnitudes, under similar conditi*

Medium	Age of culture in days	Magnitude of population samples	Spore classes according to length in microns						Constants			Spore classes according to width in microns						Constants		
			20	25	30	35	40	45	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error	3.0	3.3	3.6	3.9	4.2	4.5	Mean and probable error	Standard deviation and probable error	Coefficien of variabil and prob able erro
5 pct. potato dextrose agar	56	50	8	16	11	10	4	1	28.90 ± 0.61	6.35 ± 0.43	21.97 ± 1.38	4	15	13	10	5	3	3.64 ± 0.04	0.40 ± 0.03	10.98 ± 0.
do	56	100	33	34	16	10	4	3	26.35 ± 0.44	6.57 ± 0.31	24.93 ± 1.19	4	39	28	21	5	3	3.58 ± 0.02	0.33 ± 0.02	9.22 ± 0.
do	56	200	53	78	43	16	9	1	26.38 ± 0.27	5.65 ± 0.19	21.40 ± 0.77	9	78	74	27	8	4	3.54 ± 0.01	0.30 ± 0.01	8.47 ± 0.

TABLE 7.—*Summary of comparisons between means and coefficients of variability for length and width of 100 spores of Fusarium lini, form 1, based on data in table 6*

Conditions compared: magnitude of population sample	Length				Width			
	Means		Coefficient of variability		Means		Coefficient of variability	
	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference
50 and 100	2.55 ± 0.75	3.40	2.96 ± 1.90	1.50	0.06 ± 0.04	0.13	1.76 ± 0.86	2.04
50 and 200	2.52 ± 0.66	3.80	0.56 ± 1.65	0.33	0.10 ± 0.04	2.50	2.51 ± 0.71	3.16
100 and 200	0.03 ± 0.52	0.05	0.47 ± 1.39	0.33	0.04 ± 0.03	1.53	0.75 ± 0.52	1.43

TABLE 8.—*Variations and constants for length and width of 100 spores of Fusarium lini, form 1, on different media*

Medium	Age of culture in days	Spore classes according to length in microns							Constants			Spore classes according to width in microns						Constants		
		15	20	25	30	35	40	45	Mean and probable error	Standard deviation and prob-	Coefficient of variability and probable error	3.0	3.3	3.6	3.9	4.2	4.5	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error
5 pct. potato dextrose agar	56		33	34	16	10	4	3	26.35 ± 0.44	6.57 ± 0.31	24.93 ± 1.19	4	39	28	21	5	3	3.58 ± 0.02	0.33 ± 0.02	9.22 ± 0.44
Sweet clover	56	24	44	20	10	1	1		21.15 ± 0.34	5.09 ± 0.24	24.06 ± 1.15	10	51	29	7	3		3.43 ± 0.02	0.26 ± 0.01	7.63 ± 0.36
Peptone agar	14		8	28	33	22	7	2	29.10 ± 0.38	5.66 ± 0.27	19.50 ± 0.93	8	34	29	21	8		3.56 ± 0.02	0.33 ± 0.02	9.30 ± 0.44

TABLE 9.—*Summary of comparisons between means and coefficients of variability for length and width of 100 spores of Fusarium lini, form 1, based on data in table 8*

Conditions compared: media	Length				Width			
	Means		Coefficient of variability		Means		Coefficient of variability	
	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference
5 pct. potato dextrose agar and sweet clover	5.20 ± 0.55	9.29	0.87 ± 1.65	0.52	0.15 ± 0.03	5.35	1.59 ± 0.57	2.77
5 pct. potato dextrose agar and peptone agar	2.75 ± 0.58	4.70	5.43 ± 1.51	3.59	0.02 ± 0.02	0.83	0.08 ± 0.68	0.12
Sweet clover and peptone agar	7.95 ± 0.51	15.49	4.56 ± 1.48	3.09	0.13 ± 0.03	4.64	1.67 ± 0.57	2.91

trose culture was found to be 26.35 ± 0.44 microns; for the spores from sweet clover culture the mean length was 21.15 ± 0.34 microns. The difference between these means is more than nine times the probable error of the difference, which apparently is quite significant. The difference in the mean width of spore cultures grown on 5 per cent potato dextrose agar and on the sweet clover was found to be more than five times the probable error of the difference. This again apparently is significant. The difference in the mean length and width of spores from cultures grown on sweet clover

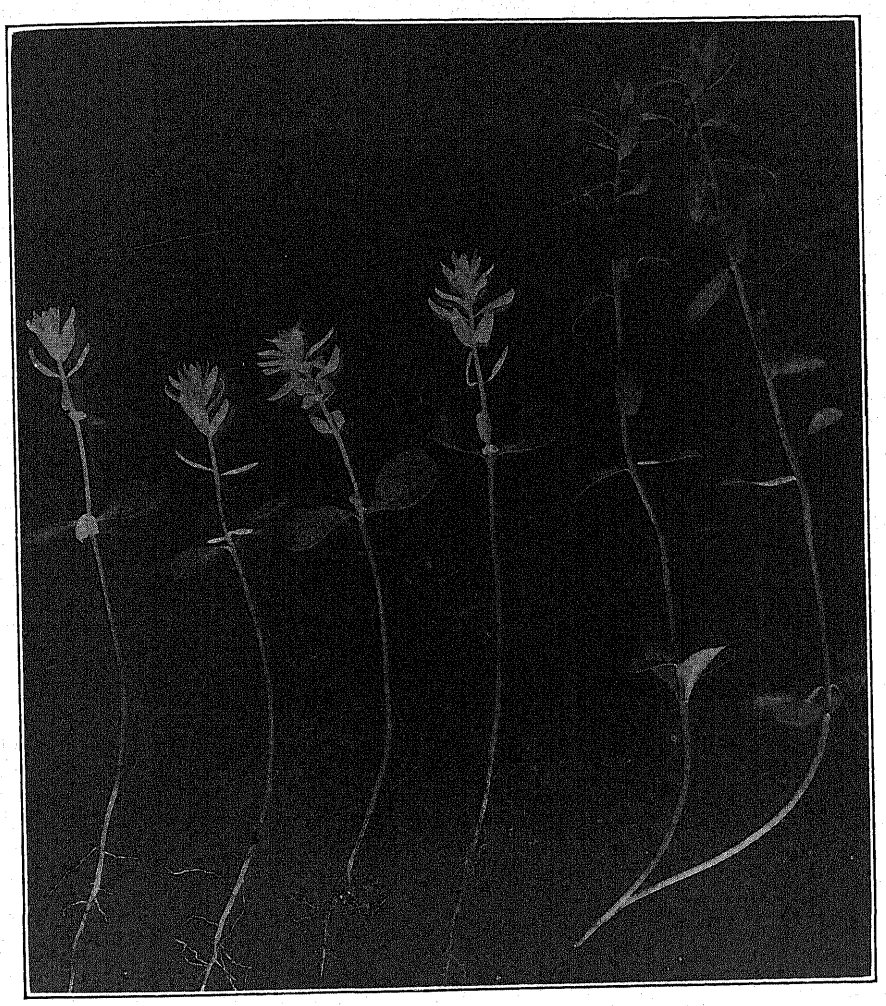

FIG. 2. Reaction to *Fusarium lini*, form 1, on individual plants of Primost, Minn. 25 (C. I. 177), taken from inoculated soil. Plants on the right are from the check pots.

and from those grown on slightly acid peptone agar were found to be respectively more than fifteen and four times the probable error of the difference. These differences also are apparently very significant, certainly with respect to the mean length. The difference in mean length and width of spores from cultures grown on 5 per cent potato dextrose agar and slightly acid peptone agar are respectively more than four times and less than one times the probable error of the difference. The difference in mean length is apparently significant; whereas the difference in width probably is not. These results are summarized in table 9. Larger or smaller differences might have been obtained if spores had been grown on other media and similarly compared. Such differences as these undoubtedly account for conflicting data on spore size given by different authors for the same organism. It is evident that there may be a marked and significant difference in spore size of a given form of *F. lini* when grown on several different media. Therefore it is important to maintain uniform cultural conditions with respect to media and environment when the comparative morphology of forms of *F. lini* is studied.

Comparisons of the mean length and width of 100 spores of nine forms of Fusarium lini from cultures grown in the same medium

Table 10 gives a summary of the measurements of nine forms from cultures 7 to 14 days old grown on slightly acid peptone agar. Form 5 was measured with a screw micrometer. The mean length varied from 20.10 ± 0.33 microns for form 7, to 35.95 ± 0.42 microns for form 5. The mean width varied from 3.27 ± 0.03 microns for form 2, to 4.08 ± 0.04 microns for form 5. These differences are apparently significant. However, forms 2 and 7 have mean lengths of 20.70 ± 0.27 microns and 20.10 ± 0.33 microns respectively; in this case the differences are smaller. Greater differences might have occurred if thousands of spores had been measured. Therefore spore size alone is not a sufficient basis for a definite separation of forms of *F. lini.*

It has been shown that the same form of *F. lini* differs in spore size when grown on different media. On the same medium, some of the forms differ significantly, while others do not. Therefore spore size of forms of *F. lini* is not an accurate criterion for separating them into distinct morphological categories; but it indicates that there are inherent differences in the spore dimensions of different physiologic forms when cultured on the same medium under identical environmental conditions.

Comparison of cultural and structural characteristics of nine forms of Fusarium lini grown on seven different media

Tables 11 to 17 inclusive summarize the structural and cultural characteristics of nine forms of *F. lini* cultured on seven media in test tubes, as

TABLE 10.—*Variations and constants for length and width of 100 spores of each of the nine physiologic forms of Fusarium lini grown on slightly acid peptone agar*

Physiologic form	Accession no.	Spore classes according to length in microns								Constants			Spore classes according to width in microns									Constants		
		15	20	25	30	35	40	45	50	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error	2.7	3.0	3.3	3.6	3.9	4.2	4.5	4.8	5.1	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error
1	3		8	28	33	22	7	2		29.10 ± 0.38	5.66 ± 0.27	19.50 ± 0.93		8	34	29	21	8				3.56 ± 0.02	0.33 ± 0.02	9.30 ± 0.44
2	7	19	54	22	4	1				20.70 ± 0.27	4.00 ± 0.19	19.30 ± 0.92		6	26	32	28	8				3.62 ± 0.02	0.31 ± 0.02	8.56 ± 0.41
3	9	2	17	57	22	2				25.25 ± 0.25	3.70 ± 0.18	14.65 ± 0.70		20	16	41	10	6	5	2		3.27 ± 0.03	0.43 ± 0.02	13.14 ± 0.63
4	8		13	24	29	32	2			29.30 ± 0.40	5.33 ± 0.25	18.19 ± 0.87		2	18	36	27	16	1			3.42 ± 0.02	0.46 ± 0.02	13.45 ± 0.64
5	2		2	7	22	24	30	14	1	35.95 ± 0.42	6.29 ± 0.30	17.50 ± 0.83	2	4	11	8	27	9	27	8	4	4.08 ± 0.04	0.61 ± 0.03	15.00 ± 0.82
6	6	4	44	48	2	1	1			23.75 ± 0.25	3.70 ± 0.18	15.60 ± 0.74			16	46	33	4	1			3.68 ± 0.02	0.24 ± 0.01	6.52 ± 0.31
7	4	37	31	28	2	1	1			20.10 ± 0.34	4.95 ± 0.24	24.60 ± 1.17		4	47	37	11	1				3.47 ± 0.02	0.23 ± 0.01	6.63 ± 0.32
8	5		1	37	50	11	1			29.70 ± 0.44	3.65 ± 0.31	12.30 ± 0.59			20	44	31	4	1			3.67 ± 0.02	0.25 ± 0.01	6.81 ± 0.33
9	1		22	39	23	12	2	2		26.95 ± 0.38	5.65 ± 0.27	21.00 ± 1.00		3	28	31	32	4	1	1		3.64 ± 0.01	0.10 ± 0.01	2.75 ± 0.13

recommended by Wollenweber *et al* (12). The percentages of spores falling in the septation classes, as listed, were obtained by examination of 100 spores as practiced by Appel and Wollenweber (2) and Sherbakoff (8). The spore sizes are given on the basis of an average of 10 macroconidia, individually measured for length and width. Spore dimensions were not given for less than 10 spores. The presence or absence of microconidia, macroconidia, terminal and intercalary chlamydospores, the color of mycelium, and substrate was quite variable. Sporulation was not common for all the forms on any one of these media. Forms 1 and 6 sporulated on only two of the media; form 4 on three media; forms 3, 5, 7, and 8 on four media; form 2 on five of the media; and form 9 on seven of the media. There was also great variation in other microscopical characters.

TAXONOMIC POSITION OF FUSARIUM LINI IN FORM GENUS FUSARIUM

Some of the characteristics common to section Elegans (12) of form genus *Fusarium* and *F. lini* are thin-walled microconidia, cylindrical to long ellipsoid, not pyriform, and not catenulate on aerial mycelium. The microconidia are usually present and dominately 0-septate. Macroconidia are usually pedicillate and attenuate at the top end. Terminal and intercalary chlamydospores are usually present. There is no blue or green color in the conidia even as a diffusion from the stroma. The stroma is principally vinaceous to lilac on artificial media.

PHYSIOLOGY OF PHYSIOLOGIC FORMS OF FUSARIUM LINI

Effect of Temperature on Rate of Growth

In order to determine whether the physiologic forms of *F. lini* used in this work could be recognized on the basis of reaction to temperature, a series of tests was made as follows:

Petri dishes of uniform size were poured uniformly with slightly acid peptone agar. At the end of two days, contaminated plates were discarded, and to the others was transferred an approximately uniform amount of mycelium from stock cultures of forms 2, 3, 4, and 5. The plates were incubated at room temperatures for two days before distribution to the various temperature incubators. At the end of 10 days, the diameter of each colony was measured and averaged. The experiment was carried out in duplicate.

The results are illustrated in figure 3. The difference in growth made by the four forms at the various temperatures evidently falls within the limits of experimental error. That is, none of the forms tested could be distinguished from each other on the basis of growth at various temperatures.

TABLE 11.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction*[d] *of nine physiologic forms of Fusarium lini grown on potato plugs under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]				Microconidia[c]	Chlamydospores[c]		Age of culture in days
		Number of septa							
		0	1	2	3		Terminal	Intercalary	
1	3	Macroconidia, if any, rare				−	+	+	30
2	7	5 −	72 20.86 x 3.33	5 −	18 30.64 x 3.33	+	−	−	34
3	9	2 −	7 −	3 −	85 38.2 x 3.52	+	+	+	51
4	8	Macroconidia, if any, rare				−	−	−	51
5	2	9 −	9 −	9 −	73 39.28 x 3.30	+	+	+	30
6	6		Rare			+	+ Rare	+	50
7	4	Macroconidia, if any, rare				−	+	+	43
8	5	80 16.32 x 3.29	19 17.78 x 3.28			+	+	+	48
9	1	93 8.39 x 1.17	7 −						

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the spore size.

[c] + indicates presence of microconidia or chlamydospores.
− indicates absence of microconidia or chlamydospores.

[d] Mycelium white throughout and color of substrate remained unchanged.

TABLE 12.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on hard oat agar under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Micro-conidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa										
		0	1	2	3	4		Terminal	Inter-calary		Mycelium	Substrate
1	3	Macroconidia, if any, rare					–	+	+	30	White to light pink	Very light pink
2	7	94 16.32 x 3.26	6 –				+	+	+	43	White	Unchanged
3	9	3 –	7 –	6 –	51 28.86 x 3.39	33 44.66 x 3.36	+	+	+	51	do	do
4	8	19 17.28 x 3.36	35 22.49 x 3.36	18 25.43 x 3.82	28 34.43 x 3.82		+	+	+	51	do	do
5	2	60 28.3 x 3.33	8 –	2 –	30 30.64 x 3.29		+	+	+	30	do	do
6	6		Rare				+	+	+	50	do	do
7	4	23 18.91 x 3.39	35 20.54 x 3.29	6 –	36 30.32 x 3.29		+	+	+	43	do	do
8	5	34 16.63 x 3.29	30 16.63 x 3.52	5 –	31 27.71 x 3.72		+	+	+	48	do	do
9	1	65 7.38 x 1.22	31 6.75 x 1.16	4 –			+	+	+	29	do	do

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the spore size.

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

TABLE 13.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on 2 per cent potato dextrose agar under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Micro-conidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa						Terminal	Inter-calary		Mycelium	Substrate
		0	1	2	3	4						
1	3	Macroconidia, if any, rare					–	+	+	31	White, light pink	Light pink
2	7	25 17.28 x 3.39	62 20.54 x 3.39	11 29.01 x 3.36			+	+	+	43	White, appressed	Unchanged
3	9	4 –	10 21.84 x 3.46	4 –	75 38.92 x 3.26	7 –	+ Rare	+	+	51	White, appressed	do
4	8	Rare	Rare	Rare			+ Very abundant	+	+	51	White	do
5	2		Rare	Rare	Rare		+	+	+	30	White, appressed	do
6	6	17 17.28 x 3.67	55 17.28 x 3.39	6 –	22 31.30 x 3.33		+	+	+	50	White	do
7	4		60 19.49 x 3.29	7 –	33 30.32 x 3.42	Rare	+	+ Very abundant	+	43	do	do
8	5	1 –	4 –	2 –	93 38.26 x 3.59		+	+	+	48	do	do
9	1	51 6.26 x 1.17	41 7.33 x 1.17	7 –	1 –		+	+	+	30	do	do

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

TABLE 14.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on 5 per cent potato dextrose agar under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa						Terminal	Intercalary		Mycelium	Substrate
		0	1	2	3	4						
1	3	3 –	5 –	9 –	83 31.3 x 4.01		+	+	+	30	White	Dark brown
2	7	Macroconidia, if any, rare					–	+	+	43	White, appressed	Unchanged
3	9	4 –	42 22.17 x 3.52	20 26.08 x 3.72	31 37.29 x 3.62	3 –	+	+	+	51	do	do
4	8	Rare	Rare				+ Very abundant	+	+	51	do	do
5	2	Macroconidia, if any, rare					+	–	–	30	do	do
6	6	Rare					+	+ Rare	+ Rare	50	White	do
7	4	Macroconidia, if any, rare					+	–	–	43	do	do
8	5	23 24.2 x 3.32	45 24.78 x 3.49	3 –	27 28.04 x 3.52		+	+ Rare	+ Rare	50	do	Very dark brown
9	1	99 7.38 x 1.22	1 –				+	–	–	30	do	Unchanged

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

TABLE 15.—*The sporulation, variation in number of septa of macroconidia, the size of macronidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on steamed rice under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]				Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa					Terminal	Intercalary		Mycelium	Substrate
		0	1	2	3						
1	3	Macroconidia, if any, rare				−	+	+	30	White	Light yellow
2	7	3 −	78 24.12 x 3.36	5 −	14 30.97 x 3.42	+	+ Rare	+ Rare	34	do	Light pink
3	9		Rare	Rare		+	+	+	51	White, appressed	Light brown
4	8	Rare	Rare			+	+ Rare	+ Rare	51	do	do
5	2	Macroconidia, if any, rare				+	+	+	30	White	do
6	6	Macroconidia, if any, rare				+	−	−	50	do	White with trace of purple at line of diffusion
7	4	Macroconidia, if any, rare				+	+	+	43	Very light brown	Unchanged
8	5	Macroconidia, if any, rare				+	−	−	45	White	White with trace of purple at line of diffusion
9	1	81 5.59 x 1.21	19 5.91 x 1.23			+	−	−	29	do	Light pink

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
− indicates absence of microconidia or chlamydospores.

TABLE 16.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color[d] reaction of nine physiologic forms of Fusarium lini grown on steamed sweet clover stems under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Microconidia[c]	Chlamydospores[c]		Age of culture in days
		Number of septa								
		0	1	2	3	4		Terminal	Intercalary	
1	3	1 –	8 –	25 23.15 x 3.49	66 22.71 x 3.62		+	+	+	30
2	7	6 –	6 –	4 –	88 32.63 x 3.39		+	+	+	34
3	9	Rare	Rare				+	+	+	51
4	8	Macroconidia, if any, rare					–	+ Very abundant	+	51
5	2	45 20.21 x 3.39	46 18.26 x 3.52	4 –	5		+	+	+	30
6	6	4 –	64 18.62 x 3.39	4 –	28 27.78 x 3.52		+	+	+	50
7	4	3 –	15 19.23 x 3.39	10 23.15 x 3.69	72 29.01 x 3.91	Rare	+	+ Very abundant	+	43
8	5	Culture dried up								
9	1	12 8.56 x 1.16	68 7.18 x 1.18	17 8.83 x 1.21	3 –		+	+	+	29

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

[d] Mycelium white throughout and color of substrate remained unchanged.

TABLE 17.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction[d] of nine physiologic forms of Fusarium lini grown on steamed alfalfa stems under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b] — Number of septa				Microconidia[c]	Chlamydospores[c]		Age of culture in days
		0	1	2	3		Terminal	Intercalary	
1	3	Macroconidia, if any, rare				−	+ Very abundant	+	30
2	7	Macroconidia, if any, rare				−	+	+	31
3	9	Rare	Rare	Rare		+	+ Rare	+ Rare	51
4	8	50[e] 17.93 x 3.33	22 21.84 x 3.29	2 −	26 28.09 x 3.33	+	−	−	50
5	2	54 26.21 x 3.39	31 29.9 x 3.39	9 −	6 −	+	+	+	30
6	6	Macroconidia, if any, rare				+	+	+	50
7	4	15 22.85 x 3.72	34 20.86 x 3.39	22 23.47 x 3.69	29 27.39 x 3.65	+	+	+	43
8	5	Rare	Rare			+	+	+	50
9	1	56 6.97 x 1.15	41 7.29 x 1.16	2 −	1 −	+	+	+	29

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
− indicates absence of microconidia or chlamydospores.

[d] Mycelium white throughout and color of substrate remained unchanged.

[e] Based on 50 spores.

TABLE 18.—*Comparative cultural characteristics of nine physiologic forms of Fusarium lini when grown on the same and different media under the same conditions*

Physiologic form	Accession no.	Medium	Mycelium								Color		Sporulation				Age of colonies in days				
			Plumose	Appressed	Cottony	Floccose	Woolly	Tufted	Silky	Cobwebby	Mycelium	Substrate	Abundant	Medium	Sparse	Absent	4	6	8	10	12
																	Size of colonies in mm.				
1	3						+				White to pink	Light brown				+	9	15	22	29	36
2	7	Potato					+				do	do	+				14	19	25	31	37
3	9				+	+	+				do	do	+				16	21	27	34	31
4	8	glucose			+		+				do	do	+				15	20	26	33	40
5	2						+				do	do	+				16	21	26	33	39
6	6	agar	+		+						do	do	+				17	23	30	37	44
7	4				+						do	Brown	+				16	22	29	36	43
8	5					+	+			+	do	Light brown	+				17	24	31	38	45
9	1				+						White	do	+				13	16	21	26	31
1	3							+			White	Brown			+		5	7	8	9	10
2	7	Prune		+					+		do	Unchanged		+			12	17	22	29	36
3	9			+					+		do	do		+			16	22	29	36	43
4	8			+					+		do	do			+		14	19	25	32	39
5	2			+					+		do	do			+		14	19	25	32	34
6	6	agar		+					+		do	do	+				14	19	26	33	40
7	4			+					+		do	Brown		+			17	23	29	36	43
8	5			+					+		do	Unchanged		+			15	20	26	32	38
9	1			+					+		do	do			+		11	14	19	24	30
1	3			+					+		White	Unchanged			+		10	17	23	29	35
2	7			+					+		do	do		+			14	19	25	31	37
3	9	Corn		+					+		do	do		+			16	22	25	36	44
4	8			+					+		do	do		+			15	20	26	33	40
5	2	meal		+					+		do	do			+		14	19	25	32	39
6	6			+					+		do	do	+				15	20	26	32	38
7	4	agar		+					+		do	do	+				14	20	26	33	40
8	5			+					+		do	do	+				15	20	26	32	38
9	1			+					+		do	do			+		12	17	22	27	33

TABLE 19.—*Comparative cultural characteristics of nine physiologic forms of Fusarium lini when grown on slightly acid peptone agar under the same conditions*

Physiologic form	Acc. no.	Color		Zoning			Mycelium				Diameter of colonies in mm. at end of eight days
		Mycelium	Substrate	Distinct	Moderate	Faint	Woolly	Cottony	Tufted	Appressed at margin	
1	3	Pink	Reddish		+			+			42
2	7	do	Unchanged			+	+			+	65
3	9	do	Light yellow			+	+			+	65
4	8	do	Unchanged			+	+			+	62
5	2	Light yellow	Light yellow			+	+			+	65
6	6	Pink	do	+			+		+		73
7	4	do	do			+		+			70
8	5	do	do	+			+		+		72
9	1	White	Cream	+			+		+		50

Effect of Medium on Cultural Characteristics

Appel and Wollenweber (2), Sherbakoff (8), and others have used the cultural characteristics of micro-organisms on different media to distinguish species of *Fusarium* which could not be differentiated on a morphological basis.

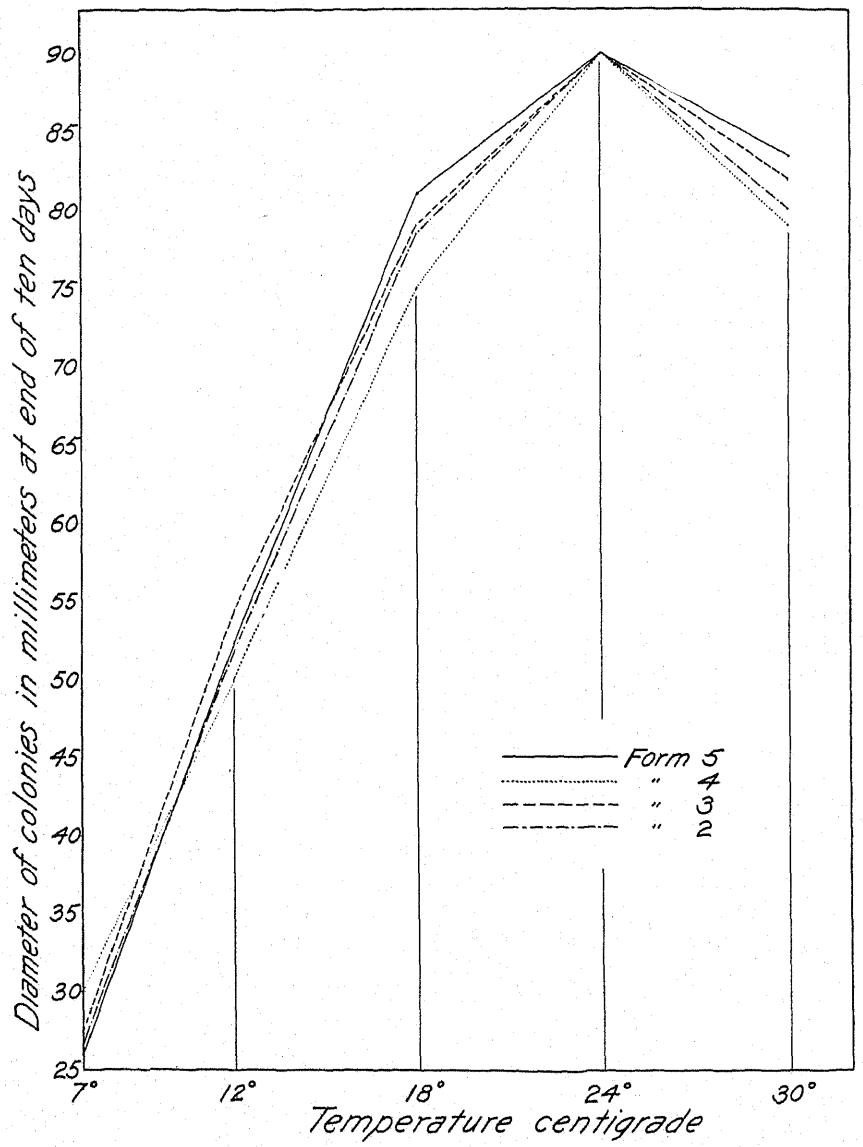

FIG. 3. Growth of four forms of *Fusarium lini* at the end of ten days on slightly acid peptone agar at various temperatures.

In order to compare their cultural characteristics, the nine forms of *F. lini* were grown on three different kinds of media: potato glucose agar, consisting of 20 grams of freshly peeled potato, 5 grams of sugar, 2 grams of agar, and 100 cc. of water; and synthetic cornmeal agar, and prune agar both manufactured by the Digestive Ferments Company, Detroit, Michigan. Triplicate plates of each medium were inoculated with small and, as nearly as possible, equal portions of mycelium of each form. Each medium was made in one lot, tubed, sterilized, and poured at the same time. All of the plates were kept under the same general environmental conditions. The nine forms listed in table 18 can be differentiated macroscopically, although with difficulty, by the following characters: color of mycelium and substrate, mycelial characters, sporulation, and rate of radial growth.

The organism which Bolley (5) described as a new species of *F. lini* was cultured on a slightly acid peptone agar. For this reason the cultural characteristics of nine forms of *F. lini* grown on slightly acid peptone agar are given in table 19.

Spore Germination

The process of spore germination in *F. lini* has been described by Bolley (5). The writer observed that one or more germ tubes, seldom more than two, are sent out from any portion of the spore, although usually from the ends. The size and number of germ tubes vary according to media and temperature.

TABLE 20.—*Summary of the percentage of germination of conidia of Fusarium lini, form 5, at various temperatures, in distilled water, with and without the addition of flax tissue, at the end of twelve hours*

Temperature in degrees C.	Flax tissue[a]	Percentage of germination				
		Trial				Average
		1	2	3	4	
7	–	16	12	10	12	12.50
	+	42	24	40	40	39.00
12	–	81	85	84	85	83.75
	+	83	90	86	90	87.25
18	–	86	84	84	82	84.00
	+	88	86	88	90	83.00
25	–	76	78	76	76	76.50
	+	82	90	86	90	87.00
30	–	71	73	74	90	90.00
	+	97	96	98	98	97.25
35	–	0	0	0	0	0
	+	0	0	0	0	0

[a] – indicates no flax tissue added; + indicates tissue added.

In distilled water at room temperatures, 20–21° C., the minimum time required for spore germination, that is, the first evidence of the germ tube protruding from the spore, was found to be 2 hours and 5 minutes in culture No. 14. When flax tissue was added to the medium, spores germinated in 1 hour and 45 minutes. The highest average percentage of germination in distilled water occurred over the rather wide range between 12° C. and 30° C. No spores germinated above 35° C., but some germinated at 7° C. At the end of 12 hours, at the lower temperatures, the germ tubes were not so long as they were at higher temperatures. Flax tissue, when added to the medium, stimulated germination greatly. These results are shown in table 20.

In tap water the spores germinated best at 30° C. In this medium also germination was stimulated when flax tissue was added (Table 21). From tables 20 and 21 it will be seen that germination was more profuse in the distilled water than in tap water.

TABLE 21.—*Summary of the percentage of germination of conidia of Fusarium lini, form 5, at various temperatures, in tap water with and without the addition of flax tissue, at the end of twelve hours*

Temperature in degrees C.	Flax tissue[a]	Percentage of germination				
		Trial				Average
		1	2	3	4	
7	–	8	6	8	6	7.00
	+	12	10	10	12	11.00
12	–	18	12	14	18	15.50
	+	70	18	16	18	18.00
18	–	18	26	30	26	25.00
	+	78	74	64	68	71.00
25	–	27	36	34	38	31.75
	+	56	74	78	60	67.00
30	–	88	90	90	78	86.50
	+	88	94	90	92	91.00
32.5	–	66	72	68	68	68.50
	+	78	74	76	72	75.00

[a] – indicates no flax tissue added; + indicates tissue added.

SUMMARY

1. Observational evidence seemed to indicate that physiologic specialization occurs in *F. lini*.

2. Experimental evidence proves that there are at least 9, and probably more, physiologic forms of *F. lini* which can be distinguished by their parasitism on the following four varieties of flax: Primost, Minn. 25 (C. I. 177);

Winona, Minn. 182 (C. I. 179); N. D. 3080 (C. I. 275); and N. D. 40013 (C. I. 241).

3. The four differential varieties vary in degree of resistance and susceptibility to seven physiologic forms.

4. Forms 1 and 2 are very virulent on all four differential hosts. Form 1, however, is quite distinct from form 2 and all other forms. When the plants inoculated with form 1 are 4–6 inches high, the green color gradually disappears from the tissues above the cotyledons until this portion of the plant is eventually colorless. The line of demarcation is very sharp between the normal and chlorotic tissues. This condition was produced by artificial inoculation in the field as well as in the greenhouse. Form 1 also causes loss of color in Manchuria barley, Victory oats, and Rosen rye. The green color gradually disappeared from the tissues above the first leaf.

5. The forms are numbered in order of their virulence, forms 1 and 2 being the most virulent, and forms 7, 8, and 9 the weakest.

6. Forms 2, 3, and 7 were found in the United States and Canada, while form 1 was found only in Saskatchewan, and the other forms in Minnesota. However, further collections might show the situation to be entirely different.

7. Variations in morphology of the spores of identical forms were found to exist under different conditions of growth. It was found necessary to measure at least 100 spores in order to obtain accurate results.

8. It was evident from this study that marked and significant differences occurred in spore size of a given form of *F. lini* when grown on several different media under the same environmental conditions. Therefore when studying the comparative morphology of forms of *F. lini* it is important to maintain uniform cultural conditions with respect to media and environment.

9. The mean length of 9 forms of *F. lini* grown on slightly acid peptone agar under uniform conditions is from 20.10 ± .33 microns for form 7 to 35.95 ± .42 microns for form 5. The mean width ranges from 3.77 ± .03 microns for form 3, to 4.08 ± .04 microns for form 5. Some of the differences are quite significant while others are not. Significant differences might have been obtained if thousands of spores had been measured. Therefore spore size of forms of *F. lini* is not a very accurate criterion for separating them into distinct morphological categories; but it indicates that there are inherent differences in the spore dimensions of different forms when cultured on the same medium under identical environmental conditions.

10. The taxonomic position of *F. lini* probably is in section Elegans of the form genus *Fusarium*.

11. No significant differences were found in the amount of radial growth of four forms of *F. lini* on the same medium at various temperatures.

12. The 9 forms can be distinguished with difficulty by macroscopic examination when grown on 3 different media under the same conditions.

13. The minimum time required for spore germination in distilled water at 20–21° C. was found to be 2 hours and 5 minutes for culture No. 14. Germination occurred in 1 hour and 45 minutes when flax tissue was added to the medium. Best germination in distilled water occurred between 12° C. and 30° C.; in tap water at 30° C. Flax tissue when added to the medium increased germination in all cases. No germination occurred above 35° C. Germ tubes of spores at 12° C. were shorter than those at higher temperatures. Some germination took place at 7° C. The percentage of germination was higher in distilled water than in tap water.

SECTION OF PLANT PATHOLOGY,
UNIVERSITY FARM,
ST. PAUL, MINNESOTA.

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PHYSIOLOGIC SPECIALIZATION AND MUTATION IN USTILAGO ZEAE[1]

J. J. Christensen and E. C. Stakman

INTRODUCTION

Corn smut, caused by *Ustilago zeae* (Beck.) Ung., apparently has been assuming increasing importance in corn production in the Upper Mississippi Valley. The disease may not be more destructive and prevalent now than it was 25 years ago; but, on account of the increase in corn production, the disease has attracted increasing attention during the past few years.

As was pointed out by Hayes et al (4), the best method of reducing losses from corn smut is the development of smut-resistant lines.[2] It is true

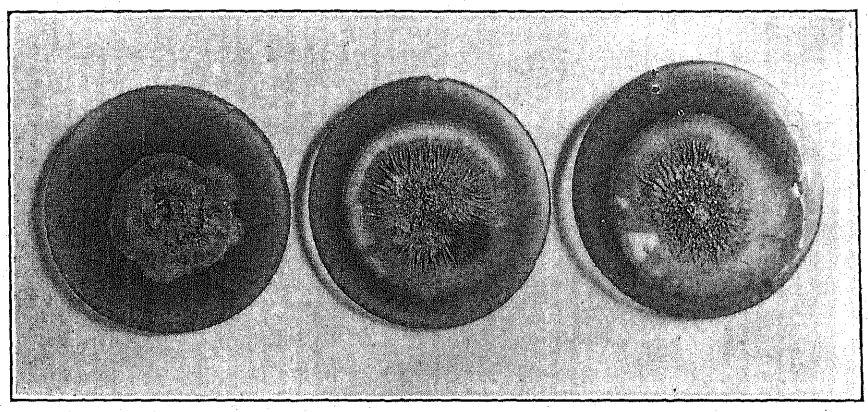

Fig. 1.—Three forms of *Ustilago zeae* showing sectors. Two or more distinct types of sectors appeared in each colony. From left to right: form 7 from Massachusetts; form 12 from New Hampshire; and form 1b from Minnesota, which itself was originally isolated from a sector.

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[2] For several years the Section of Plant Breeding and the Section of Plant Pathology of the University of Minnesota have cooperated on a project for the development of smut-resistant lines of corn. The investigation reported in this paper was made as a part of the general project.

that the destructiveness of the disease probably can be reduced somewhat by good cultural practices, sanitation, and proper soil fertilization. However, the only really adequate method of reducing losses would seem to be the use of resistant lines. Jones (5), Hayes et al (4) have demonstrated the possible feasibility of this method of controlling the smut. However, there is always the lingering possibility in developing resistant lines that they may remain resistant only as long as they are exposed to infection by the particular physiologic form or forms of the pathogene to which they were exposed during the process of their creation.

For several years there has been circumstantial evidence of the existence of physiologic forms of *U. zeae*. As a result of extensive observations, Melchers (6) pointed out, in 1920, the strong probability of the existence of forms of the pathogene. More recently, Stakman and Christensen (10) definitely demonstrated the existence of several forms. Tisdale and Johnston (12) also noted a difference in pathogenicity of different strains of the organism.

It seemed highly desirable, therefore, to make a thorough study of the number, characteristics, and geographic distribution of physiologic forms

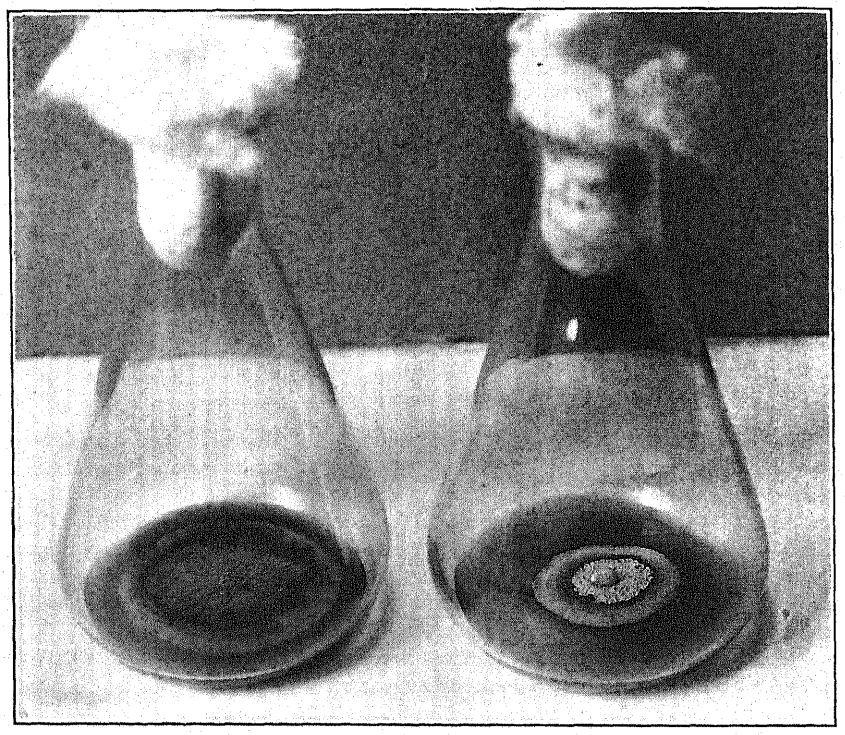

FIG. 2.—Mutant and its parent. Left, form 6a; right, parent form 6.

of *U. zeae* in the United States. It obviously is highly important also to ascertain whether these forms are relatively stable or whether they change rapidly. For the past two years, therefore, investigations have been made on these phases of the problem at the University of Minnesota.

MATERIALS AND METHODS

Twelve collections of *U. zeae* were obtained in 1924 and 1925, from the sources indicated in table 1. Cultures were obtained as follows: spores were

TABLE 1.—*Source of 15 physiologic forms of Ustilago zeae*

Form	Origin	Locality	Year	Collector
1	Smut boil	Minnesota (Isanti)	1925	Writers
1b	Sector from form 1	.....	1925	.....
2	Smut boil	Minnesota (University Farm)	1924	Writers
3	do	Manitoba, Canada	1924	D. L. Bailey
4	do	Nebraska	1925	A. F. Thiel
5	do	Ohio	1925	H. A. Rodenhiser
6	do	Texas	1925	W. Butler
6a	Sector from form 1	.....	1926	.....
7	Smut boil	Massachusetts	1925	H. A. Rodenhiser
8	do	Pennsylvania	1924	P. Brierley
8a	Sector from form 8	.....	1925	.....
9	Smut boil	Colorado	1925	A. E. Lungren
10	do	Mississippi	1924	J. M. Wallace
11	do	Maine	1925	D. Folsom
12	do	New Hampshire	1925	H. A. Rodenhiser

taken from a smut ball, from the inside whenever possible, and kept for 24–72 hours in a 1 per cent solution of copper sulphate, which was shaken from time to time in order to insure wetting of the spores. Some of the spore suspension was then removed from the container by means of a sterile platinum loop, and streak inoculations were made on a 2 per cent potato dextrose agar in petri dishes. Pure cultures often developed from this first inoculation. Transfers then were made, and stock cultures were kept at room temperature in the laboratory.

In order to compare the cultural characters of forms obtained from the different collections, transfers were made to several different media in triplicate petri dishes, flasks, or tubes. For these comparative studies, the agar always was made up in one lot, tubed, sterilized, and poured at about the

same time. After inoculation, the containers were kept at room temperature on the same table in the laboratory, in order that all might be subjected to the same general environmental conditions.

In order to obtain sporidia for inoculation, transfers were made from the different cultures to a 1 per cent carrot-dextrose decoction. Sporidia were produced in great abundance in this nutrient medium. Just before

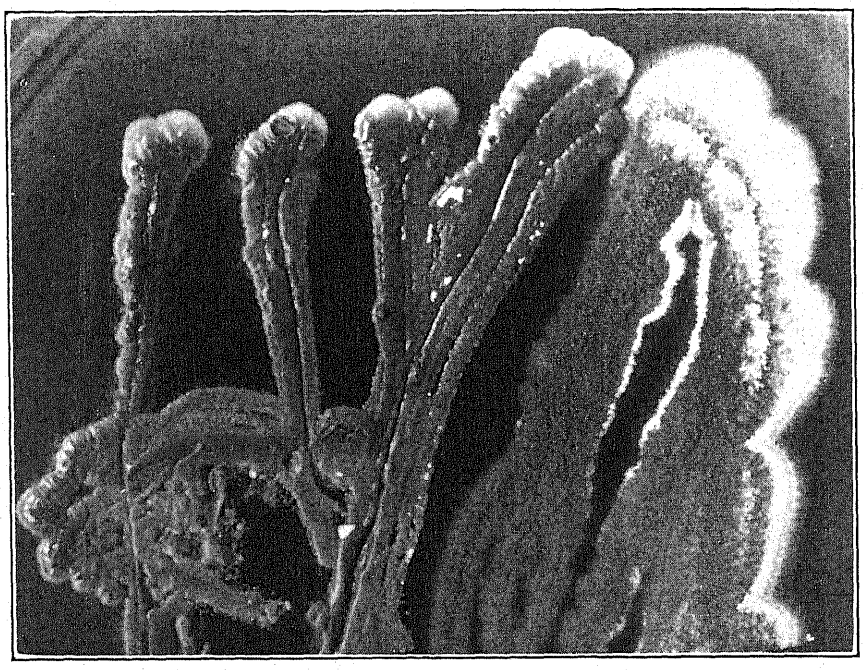

FIG. 3.—Two physiologic forms of *Ustilago zeae* growing in the same petri dish. On the left, form 6 from Texas; on the right, form 1 from Isanti, Minnesota.

the material was used for inoculating plants, an equal amount of fresh nutrient was added to each of the flasks.

Inoculations were made on selfed lines of corn[3] in the greenhouse and in the field. Two methods were used in the greenhouse: (a) numerous punctures were made with a sterile needle in the stalk of the corn plants, which were then sprinkled with a suspension of conidia; (b) about 1 to 1½ cc. of the suspension of sporidia was injected, with a hypodermic needle, into each stalk. Only the latter method was used in the field. Control plants, of course, always were punctured or injected with the sterile nutrient solution.

[3] All selfed lines of corn were furnished by the Section of Plant Breeding of the University of Minnesota. A selfed line of teosinte was obtained from the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

RESULTS

Cultural Characters of Different Forms.—Fifteen distinct forms were obtained, either directly from the original collections or as mutants from certain forms. There are striking and consistent cultural differences between the different forms. They easily can be differentiated on culture media by the following characters: rate of growth, color, relative amount of aerial mycelium, nature of mycelial growth, topography, zonation, surface, character of the margin, and abundance of conidia. The data on cultural characters are summarized in table 2.

The cultural characters of a given form are remarkably constant on the same medium and under the same general environmental conditions. However, they may differ greatly on different media and under different conditions of light, temperature, etc. Different forms sometimes look almost exactly alike on one medium but are entirely different in appearance on another. It should be emphasized, therefore, that cultural differences between forms can be detected only if the proper differential materials are used. And it may be worth while emphasizing the fact that there is a great deal of variation in the cultural characters of the same form under different conditions. Similar facts have been observed for various smut fungi by Sartoris (9), and by Christensen (1 and 2) for *Helminthosporium sativum* P. K. and B. The differences in cultural characters of different forms of *U. zeae* are sometimes far greater than those between the so-called species, *Ustilago tritici* (Pers.) Rost. and *U. nuda* (Jens.) K. and S., which, as Rodenhiser (8) recently has shown, are probably themselves only physiologic forms, and both of which, if one does consider them as separate species, contain many culturally distinct forms. Furthermore, some forms of *U. zeae* differ from each other more than they differ from certain forms of *U. nuda* and *U. tritici*. The writers also have observed such cultural differences between different collections of *Sorosporium reilianum* (Kühn) McAlpine.

There can be no question whatever regarding the distinctness of different physiologic forms in culture. (See Figs. 1–3.) Naturally, however, the most important question is, are these forms pathogenically distinct? And particularly important is the question as to whether any of them are so virulent as to be able to infect normally lines of corn which have been resistant in field experiments. Experiments were made to answer these questions.

For the field tests of comparative virulence of forms, ten lines of corn, which had been selfed from five to seven years, were selected. These lines had been grown for several years in the experimental plots at University Farm, St. Paul, where they were inoculated artificially each year. Their reaction to the form or forms of *U. zeae* at University Farm was therefore

TABLE 2.—(*Continued*)

Form	Age in days	Diameter of colonies in mm.	Average radial growth per day	Color	Topography	Surface	Amount of aerial mycelium	Zonation	Margin
6	20	29.6	1.5	Outer part brownish	Convex, umbonate; few shallow reticulations	Center powdery; margin china-like	0	Sharp near the edge	Not filamentous, undulate
6a	12	34.3	2.9	Purple-brown	Verruculose in center, grading to a plane surface	Velvety	++	None	Filamentous, slightly undulate
	20	53.0	2.6	Center gray, grading to dark purple	Rugose, wrinkled, ridged, except ray portion	Powdery to velvety	++	None	do
7	12	25.6	2.1	Cream to brown, scattered white patches	Raised to umbonate, slightly wrinkled	China to powdery	+=	Moderate (Margin only)	Slightly wavy to lobate
	20	32.3	1.6	do	An increase in wrinkles; sectors present	do	+=	do	do
8	12	25.6	2.1	White to tan	Convex, plane; rugose in spots	China-like	+=	Faint	Undulating in parts, myceloid
	20	38.5	1.9	do	Convex, plane; rugose in spots, sectors present	do	+=	do	do
8a	12	35.5	2.9	Center tan, rays white	Umbonate and appressed, border plane	Velvety to felty	++	None	Myceloid, slightly undulate
	20	50.5	2.5	do	do	Velvety	++	Moderate	do
10	12	41.3	3.4	Light purple	Reticulated, convex to umbonate	Center dull, edge waxy	0	None	Deeply lobed
	20	54.5	2.7	Light purple, grayish margin	Reticulated, convex to umbonate, appressed margin	Edge velvety	+ (Margin only)	Prominent (Margin only)	Deeply lobed, myceloid

TABLE 2.—*Cultural characters of 12 forms of Ustilago zeae grown under identical conditions on potato dextrose agar in Erlenmeyer flasks*

Form	Age in days	Diameter of colonies in mm.	Average radial growth per day	Color	Topography	Surface	Amount of aerial mycelium	Zonation	Margin
1	12	36.0	3.0	Deep tan to brown, grading to cream at margin	Umbonate, wrinkled and ridged	Velvety	++[a]	None	Filamentous, slightly undulate
	20	54.6	2.7	Deep purple to brown	do	do	++	None	do
1b	12	25.0	2.0	Cream to light tan	Convex to umbonate; wrinkled and radially ridged	Dull china-like to velvety	+	None	do
	20	39.1	2.0[a]	Cream to light tan; white margin	Same, but more pronounced	Dull china-like to velvety, becoming powdery	+	None	do
2	12	31.0	2.5	Whitish, grading to yellow brown	Hummocky in center to striate on ray portion	China-like to powdery	+–	None	do
	20	42.0	2.1	Purplish brown	Sharply radially ridged, except in center and outside ray	Powdery to velvety	+	Faint	do
3	12	34.2	2.8	Center brown; margin white; remainder tan	Plane, convex to umbonate	Powdery to felt-like	++	None	do
	20	55.0	2.8	Center grayish, powdery	do	do	++	Moderate	do
6	12	24.0	2.0	Center gray-brown; margin yellowish white	Convex, umbonate; few shallow reticulations	Center powdery; margin china-like	0	Sharp near the edge	Not filamentous, undulate

TABLE 2.—*(Continued)*

Form	Age in days	Diameter of colonies in mm.	Average radial growth per day	Color	Topography	Surface	Amount of aerial mycelium	Zonation	Margin
11	12	35.3	2.9	Gray-brown	Convex, plane, slightly wrinkled in patches	Powdery	++	None	Filamentous, undulate
	20	56.3	2.8	Gray-purple	do	do	++	Slight	do
12	12	32.3	2.6	Whitish-yellow to brown	Convex, papillate in center	China-like to powdery	+−	None	do
	20	49.0	2.5	Center cream to lilac, margin white	Convex, papillate in center; sectors present	Velvety	+−	Slight	do

[a] ++, abundant; +, moderate; +−, slight; +=, trace; 0, absent.

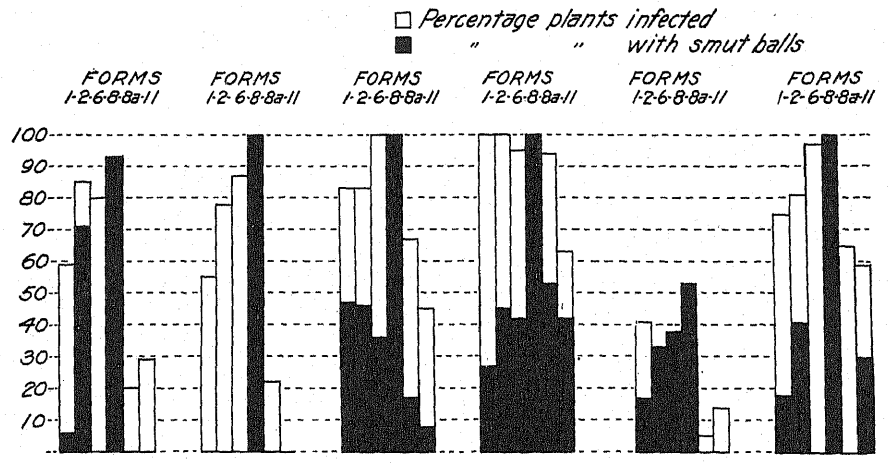

FIG. 4.—The reaction of selfed lines of corn to six physiologic forms of *Ustilago zeae*. Strains of corn, left to right, are: Longfellow (Culture 700), Longfellow (Culture 6000), Rustler (Culture 4400), Rustler (Culture 3300), N. W. Dent (Culture 4100), Brown Aleurone (Culture 1700).

known. Resistant (low smut), susceptible (high smut), and intermediate lines were selected.

One long row of each line was planted early in June. The rows were 3½ feet apart and the hills were 1 foot apart in the rows. The plot then

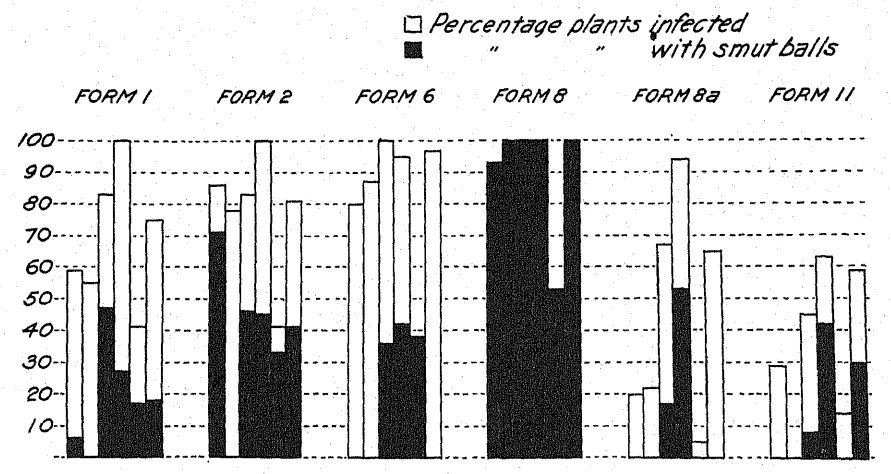

FIG. 5.—The reaction of selfed lines of corn to six forms of *Ustilago zeae*. For each physiologic form, the six lines of corn, from left to right, are: Longfellow (Culture 700), Longfellow (Culture 6000), Rustler (Culture 4400), Rustler (Culture 3300), N. W. Dent (Culture 4100), Brown Aleurone (Culture 1700).

was subdivided into 12 subdivisions, each containing from about 20 to 30 plants of each selfed line. The plants in each subdivision were inoculated on July 9, and again on July 24, with one of the physiologic forms of *U. zeae*. As previously mentioned, all inoculations in the field were made with a hypodermic syringe.

The first notes were taken seven days after the first inoculation. All of the forms caused infection and most of the plants had become infected, except the controls, which were entirely free from smut. The first evidence of infection was the appearance of chlorotic areas, two or three days after inoculation. Necrosis often followed the chlorosis, the degree and extent depending on the line of corn and the particular form of the fungus with which it had been inoculated. There were no signs of chlorosis nor other evidences of infection on the controls, except on a few plants which became naturally infected late in the summer. By August 9 there were large smut boils on many plants, while others apparently had outgrown the infection.

There were striking differences, corresponding with those observed in the greenhouse, in the pathogenicity of different forms (Table 3 and Figs.

FIG. 6.—Left: Salmon Silk (Culture 1800—1925); only very small smut boils developed. Right: Brown Aleurone (Culture 1700—1925): note large smut boils and distortion of plants. Of 21 plants inoculated in field, 20 were killed.

4 and 5). At least 7, and probably 8, of the 12 forms with which inoculations were made were decidedly different. Some of them differed from each other in their effect on all 10 of the lines of corn, while others differed only in their action on a few of them.

The differences in the effect of the different forms sometimes were very sharp. Certain forms produced only very minute smut boils on some lines of corn; others produced very large smut boils on nearly all lines; and still others characteristically caused very sharp chlorosis on many lines. In fact, there were remarkably sharp differences in the effect of the different forms. Some were extremely virulent, others moderately so, and still others only weakly pathogenic. (See Figs. 6–8.)

Form 8, from Pennsylvania, was the most virulent of all. This form infected nine of the ten selfed lines of corn heavily, and produced large smut boils on practically all plants in each plot. It not only caused heavy infection on susceptible lines of corn but also on some which had been resistant at University Farm for several years. For instance, it killed outright 20 of the 21 inoculated plants of Rustler, culture 4400, a "resistant line." The only line of corn resistant to this form was Salmon Silk, culture 1800. (See Figs. 6, 9, 10.)

Form 6, from Texas, easily could be distinguished from all the other

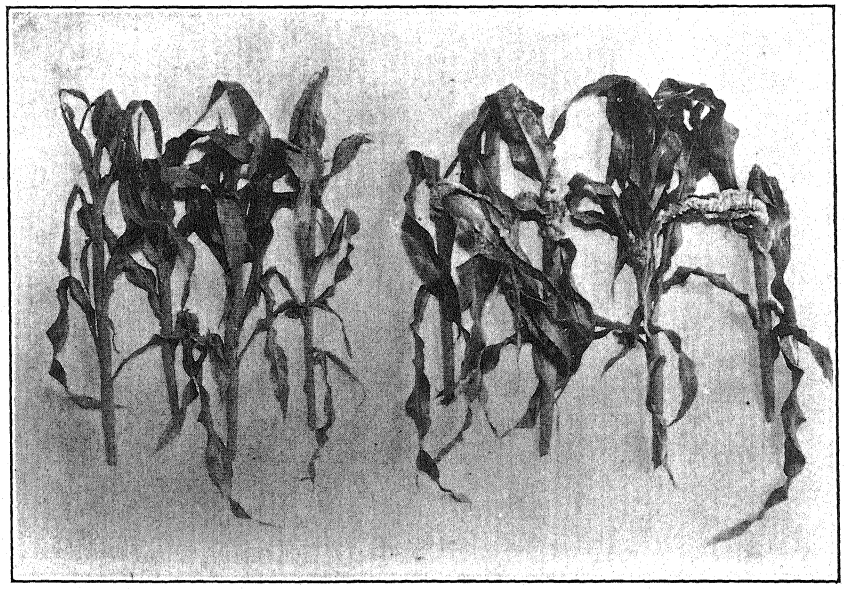

FIG. 7.—The effect of two physiologic forms of *Ustilago zeae* on a "resistant" line of corn, Minnesota 13 (Culture 4000—1925), selfed five years. On the left, form 8a obtained from a sector from form 8 on the right.

forms because of the extensive and pronounced chlorosis which is caused on several of the lines of corn.

Form 7, from Massachusetts, was the weakest. Only one line of corn, Rustler, culture 3300, was susceptible to it. On all other varieties the only indication of infection was a slight chlorosis and, occasionally, small smut boils.

It is quite obvious from the results that many of the lines of corn which have been highly resistant in the regular field tests of previous years were completely susceptible to some of the forms of the smut fungus. On the other hand, some of the susceptible lines of corn were resistant to the weakest of the forms. It is quite clear from the results that one is not justified in speaking of resistant or susceptible lines of corn unless one specifies the particular forms of the pathogene involved. For instance, Longfellow, culture 6000, was resistant to 11 of the 12 forms, but it was quite susceptible to form 8.

Stability of the Physiologic Forms.—The range of variability of forms of *U. zeae* is very wide. The character and the rate of growth, the ability to produce sporidia, and the morphology of the organism are influenced profoundly by the kind, proportion, and amount of food available, and by other

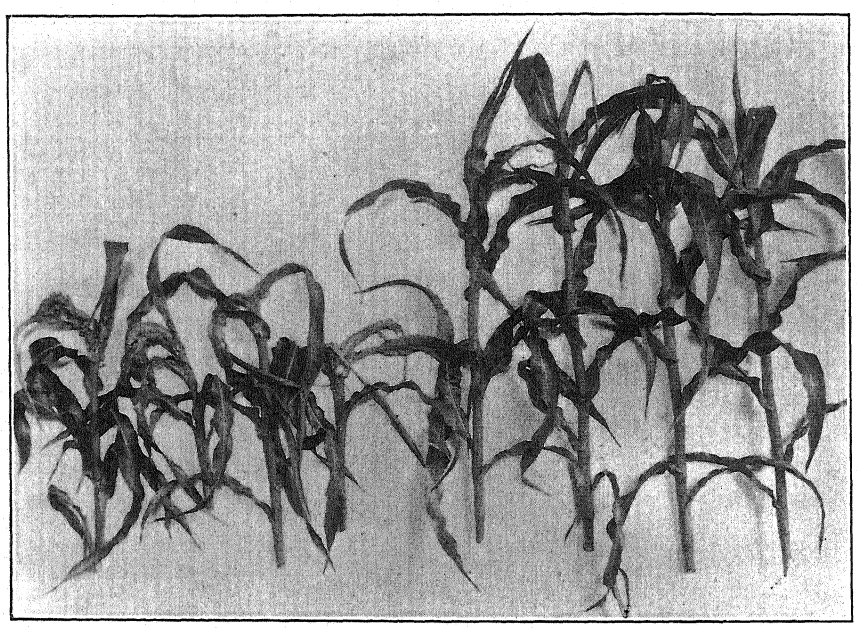

FIG. 8.—The effect of two physiologic forms of *Ustilago zeae* on a "resistant" line of corn, Rustler (Culture 4400—1925), selfed six years. On the left, form 8; right, form 7.

TABLE 3.—*Results of inoculating 10 selfed lines of corn in the field with 12 physiologic forms of Ustilago zeae*

Form	King Phillip Culture 6200 Susceptible[a]						Longfellow Culture 6000 Moderately resistant						Longfellow Culture 700 Resistant						Rustler Culture 3300 Susceptible						Rustler Culture 5800 Moderately susceptible					
	No. plants inoculated	Percentage infected	Necrosis[b]	Chlorosis[b]	No. plants with boils	Abundance and size of boils[b]	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils
1	15	100	n	c	3	1 f	18	55	n=	c=	0	–	17	59	n=	c=	1	2 f–	15	100	n–	c+	4	1 to 5 f	19	83	n–	c–	2	2 f
1b	20	95	n+	c+	3	1= f	18	6	n=	c=	0	–	15	93	n–	c=	0	–	18	83	n–	c	4	1to4 f	20	90	n–	c–	1	4 f
2	18	100	n	c	18	1=to1+ a	18	78	n–	c	0	–	14	86	n=	c–	10	3 m	20	100	n–	c+	9	1to4 a	18	100	n=	c	6	1to4 f
3	14	72	n=	c–	2	1– f	19	16	n	c	1	1 f	14	78	n–	c–	0	–	16	93	n=	c+	7	1to4 f	29	72	n=	c=	3	3 f
6	13	100	n–	c	1	1= f	16	87	n=	c=	0	–	15	80	n=	c	0	–	19	95	n–	c++	8	1to3 f	20	85	n–	c–	3	1to3 f
6a	18	83	n–	c–	1	1= f	18	33	n=	c=	0	–	14	29	n=	c=	0	–	18	61	n–	c++	3	1to4 f	20	75	n=	c=	4	1to3 f
7	19	0	–	–	–	–	12	0	–	–	–	–	17	0	–	–	–	–	14	64	n=	c=	9	1to5 m	18	17	n=	c=	0	–
8	16	100	n–	c+	16	2to4 a	18	100	n=	c–	18	2to3 a	15	93	n=	c=	14	1to5 a	16	100	n–	c–	16	1to5 a	23	100	n–	c	23	2to5 a
8a	15	40	n=	c=	2	1= f	18	22	n=	c=	0	–	15	20	n=	c=	0	–	17	94	n=	c	9	1to4 f	29	74	n–	c–	2	2– f
10	10	30	n=	c=	2	1to2 f	20	45	n=	c–	1	1= f	10	60	n=	c=	0	–	18	78	n=	c=	9	1to4 m	27	24	n=	c=	0	–
11	16	75	n=	c–	2	1= f	17	0	–	–	–	–	14	29	n=	c=	0	–	19	63	n–	c–	8	1to4 f	18	55	n=	c=	4	1to5 f
12	7	100	n	c	2	1= f	33	13	n=	c=	0	–	6	50	n=	c=	0	–	17	94	n=	c–	3	1to2 f	18	67	n–	c–	0	–
Con-trol	20	0	–	–	–	–	17	6	0	0	0	–	15	0	0	0	0	–	15	40	n=	c=	6	1to4 f	20	15	n⁰	c⁰	3	2to4 f

TABLE 3.—(*Continued*)

Form	Rustler Culture 4400 Resistant						Minn. No. 13 Culture 4000 Resistant						N. W. Dent Culture 4100 Resistant						Brown Aleurone Culture 1700						Salmon Silk Culture 1800					
	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils
1	30	83	n =	c	14	1 to 5 a	17	100	n =	c =	0	–	29	41	n –	c =	5	1 to 5 f	28	75	n =	c –	5	1 to 4 f	19	43	n =	c =	0	–
1b	29	97	n –	c	3	1 to 3 f	15	87	n =	c –	0	–	29	100	n –	c ++	0	–	29	90	n	c	10	1 to 5 m	18	28	n =	c =	0	–
2	24	83	n –	c –	11	1 to 3 a	14	93	n =	c –	9	1 to 2 f	30	33	n =	c =	10	1 to 2 f +	27	81	n =	c –	11	1 to 4 m	21	62	n –	c	0	–
3	18	67	n =	c =	0	–	26	81	n =	c –	0	–	19	0	–	–	–	–	17	41	n =	c =	2	1 to 3 f	24	41	n –	c –	0	–
6	28	100	n =	c ++	10	1 to 3 f	16	100	n –	c ++	2	1 to 3 f	29	38	n =	c =	11	1 to 3 m	28	97	n =	c +	0	–	17	88	n =	c	0	–
6a	26	65	n	c –	3	1 to 2 f	16	93	n =	c =	0	–	27	22	n =	c =	0	–	29	75	n =	c –	7	1 to 2 f	19	42	n –	c =	0	–
7	29	50	n =	c =	2	1 to 3 f	13	46	n =	c =	0	–	29	0	–	–	–	–	27	26	n =	c =	6	1 to 5 f	19	37	n =	c =	0	–
8	21	100	n =	c	21	1 to 5 a	15	100	n =	c –	15	1 to 5 a	19	53	n =	c =	10	1 to 4 m	21	100	n =	c –	21	1 to 5 a	15	80	n =	c –	9	1–to 2[c] a
8a	18	67	n =	c –	3	1 to 2 f	20	100	n =	c	3	1 to 2 f	18	5	n =	c =	0	–	17	65	n =	c	3	1 to 4 f –	22	69	n =	c =	0	–
10	11	72	n =	c =	0	–	27	85	n =	c =	1	1 f	10	0	–	–	–	–	19	15	n =	c =	2	1 to 3 f –	27	37	n =	c =	0	–
11	24	45	n =	c =	2	1 to 4 f –	17	29	n =	c =	1	1 + f	29	14	n =	c =	0	–	27	59	n =	c =	8	1 to 5 f +	19	32	n =	c =	0	–
12	10	70	n =	c =	0	–	23	–	–	–	–	–	10	–	–	–	–	–	30	–	–	–	–	–	20	–	–	–	–	–
Control	27	4	0	0	1	1 = f =	15	0	–	–	–	–	29	0	–	–	–	–	27	3	0	c =	3	1 to 4 f	20	0	–	–	–	–

environmental conditions. Comparative cultural tests made during the past two years indicate that some physiologic forms remain constant and always appear the same under identical conditions. Temporary phenotypes, or modifications, induced by differences in environmental conditions, occur frequently, although the genotype remains unaltered. Such variants revert to the parental phenotype as soon as the causal stimulus is removed.

However, some forms mutated frequently, if one can designate as mutants the wedge-shaped sectors which often occur in cultures. There was mutation in forms obtained from eight different states. Of all forms studied, only four failed to produce any mutants. Some of the forms mutated much more readily than others. Form 12, for instance, produced at least three distinct mutants in the same culture.

That these so-called mutants really are new genotypes, and not mere phenotypes caused by environmental conditions, is indicated by the fact that they bred true during repeated subsequent transfers. Some of these mutants have been grown on many different kinds of media, and under different conditions, for a considerable period of time without losing their distinctive character. Form 8a has been cultured for 1½ years and form 6a for about 10 months, and during this time they have remained constant.

Some of the mutants again mutated. Thus, from a single isolation made from a smut boil collected at Isanti, Minnesota, six distinct mutants were obtained, all of which retained their distinctive characters after repeated transfers (Fig. 11). Form 1bba (Fig. 11) was obtained through a series of three successive and distinct sectors, and a culture of this form produced three other distinct sectors. It is possible that the last three may be variants rather than mutants, as they have not been studied very long. It may be noteworthy that the last sector obtained from this series of cul-

TABLE 3.—(*Continued*)

[a] The terms "susceptible," "moderately resistant," and "resistant" indicate the reaction of the selfed lines of corn to smut under field conditions at University Farm when inoculated artificially with *U. zeae* from University Farm.

[b] Explanation of symbols:

n and c denote the degree of necrosis and chlorosis, respectively.

a indicates an abundance of smut boils.

m indicates a moderate number of smut boils.

f indicates few boils.

A plus or minus sign further differentiates the class within the respective groups.

The numerical values 0 to 5 indicate the relative size of the smut boils. 0 indicates absence of boils; 1 indicates boils varying from warts to ½ inch; 2 = ½ to 1 inch; 3 = 1 to 2 inches; 4 = 2 inches to 3 inches; 5 = 3 inches or above.

[c] Mostly 1 infections.

tures, *i.e.*, 1bba, differs much more from its parent, form 1, than 1a or 1b which developed earlier in the series of cultures.

The possibility is not excluded that these sectors which have been designated as mutants may arise from normal segregation. Sartoris (9) states that sporidia of *U. zeae*, when placed on glycerine agar, germinate and produce germ tubes which conjugate. A binucleate mycelium is thus formed. However, the shape of the sectors, and their mode of origin, suggest mutation rather than segregation. The phenomenon is similar to what has been interpreted as mutation in many other fungi. A fuller study is necessary, however, before a definite statement can be made. But, from the pathological standpoint, the essential fact is that sudden and permanent changes occur in physiologic forms of *U. zeae*. The most important question naturally is: is there a change in pathogenicity corresponding with a change in appearance?

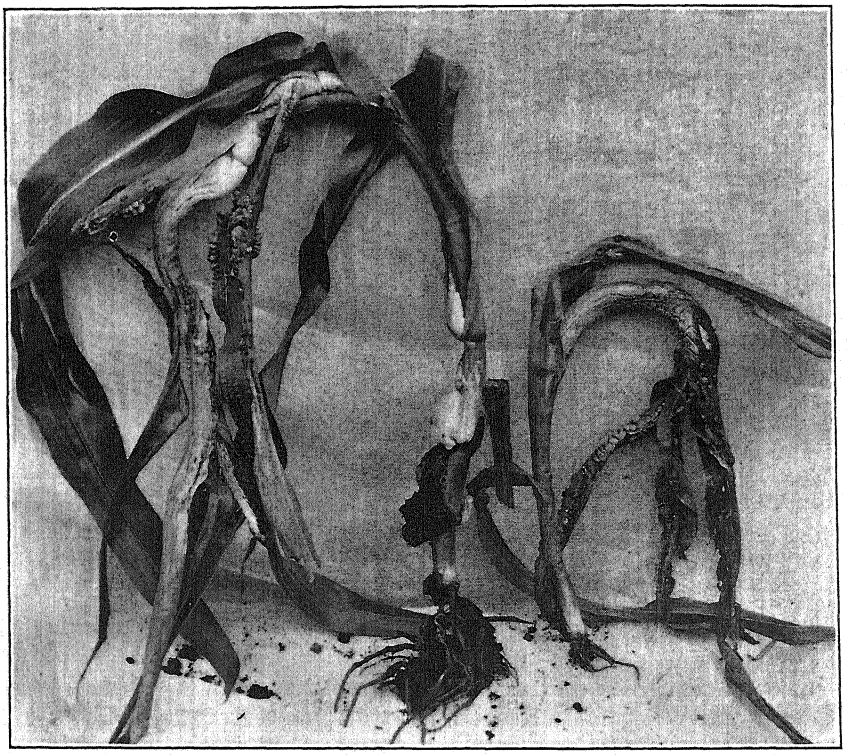

FIG. 9.—A "resistant" line of corn, Rustler (Culture 4400—1925), inoculated with form 8, from Pennsylvania. This was the typical appearance in the plot of all the plants of the line.

Pathogenicity of Mutants.—In order to ascertain whether mutants were pathogenically different from their parents, three of them were included in the field experiments already described. Mutants 8a, 6a, and 1b were used. All ten lines of corn were inoculated with them. Mutant 1b was approximately as virulent as its parent; 6a was slightly less virulent than its parent, while 8a, which was obtained from the most virulent of all forms studied, was decidedly less virulent than its parent. In fact, the latter mutant seemed to have lost its pathogenicity almost completely. There was a tremendous difference between the pathogenicity of its parent and that which it itself showed. (See table 3 and fig. 7.)

There is further evidence on the constancy of mutants from the inoculation experiments. Form 8 and its mutant 8a have been transferred approximately the same number of times in culture and have been grown under many different environmental conditions, yet after 21 months their cultural characters are still distinct, and their pathogenic capabilities remained the same after at least six months in culture.

The loss in virulence indicated above can not be attributed to attenuation in culture, because forms 2 and 8 are very virulent, while forms 7 and

FIG. 10.—Two selfed lines of corn severely injured by a virulent form of *Ustilago zeae*, form 8, from Pennsylvania. Front row, Longfellow (Culture 700—1924). Back row, Rustler (Culture 3700—1925). Both strains had previously proved resistant to *U. zeae* for several years at University Farm.

12 are weakly pathogenic, although the first two have been cultured at least a year longer than the two latter.

The fact may or may not be significant that none of the mutants tested were more virulent than their parents. However, Christensen (2) has shown that mutants of *Helminthosporium sativum* sometimes are more virulent than their parents, and it is entirely possible that the same thing may prove to be true of *U. zeae*.

DISCUSSION AND CONCLUSIONS

There are many physiologic forms of *U. zeae* which differ greatly in cultural characteristics as well as in pathogenicity. It is unfortunate but true that many supposedly resistant lines of corn probably are resistant in restricted localities and for limited periods of time only. This is clearly shown because of the fact that selfed lines of corn which had been highly resistant for several years at University Farm were completely susceptible to forms obtained from other states. It also is significant that several different forms of *U. zeae* may occur within a rather restricted geographical locality. For instance, one form was obtained at University Farm, St. Paul, and another rather distinct one was collected approximately 50 miles away.

The fact that forms mutate makes it rather difficult to be sure of the situation in breeding resistant lines, because the situation is constantly

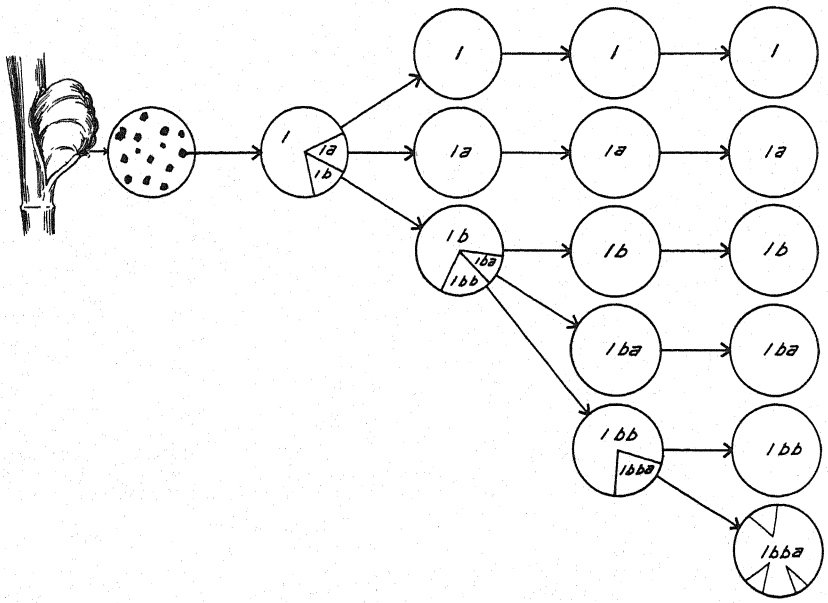

FIG. 11.—Diagrammatic representation of the origin of five distinct variants through successive sectoring in culture media from a single isolation of *Ustilago zeae*.

changing. It is to be hoped that the mutants of *U. zeae* are always less virulent than their parents. But there is no assurance and little hope, from what is known of other fungi, that this is true. It seems likely that breeding for smut resistance must be done for certain localities, and it is likely that it may have to be done more or less continuously. In view of the fact that there are many and changing physiologic forms of the pathogene, the corn smut problem probably can not be solved easily, once and for all. It probably will be a contest between a pathogene which is continually shifting its base, and breeders who are trying to keep a few steps ahead of the pathogene. How many physiologic forms there are, what their distribution is, and exactly how much they will complicate the breeding problem remains to be seen. The writers make no predictions. This much, however, is certain: there are physiologic forms of *U. zeae*, and lines of corn which have been resistant for several years to certain physiologic forms are completely susceptible to others. A line may be resistant in one region and completely susceptible in another; and it may be relatively free from smut in one locality for some time, and then become heavily infected on account of the advent of a form of smut to which it is susceptible.

Whatever the ultimate importance of physiologic specialization may prove to be, it is essential to take the phenomenon into consideration in breeding work. Information must be obtained regarding the number of physiologic forms, their geographic distribution, their degree of constancy, their pathogenic capabilities, and the degree of mobility.

The question naturally arises as to how rapidly a virulent form may be disseminated if it occurs only in a restricted locality. Theoretically it should be possible for the fungus to spread very rapidly. Almost inconceivably large numbers of sporidia are formed; they retain their viability under adverse conditions for months, as Piemeisel (7) has shown; and they have been caught several thousand feet high in the air (11).

While the problem of developing resistant lines of corn is complicated by the existence of physiologic forms, breeding work will still be valuable and may eventually solve the corn smut problem. However, the methods of developing the resistant lines must be changed. It will be necessary to inoculate them with all of the forms which occur in a region for which the varieties are intended, and it may be necessary to resort to more crossing and recrossing in order to develop resistant lines synthetically, as is now being done in developing varieties of wheat resistant to stem rust (3).

SUMMARY

1. *Ustilago zeae* is a group species comprising numerous physiologic forms.

2. The writers studied fifteen forms in considerable detail. The forms can be distinguished in culture by the following characters: rate of growth, color, topography, surface, zonation, conidial production, and margin.

3. At least seven, and possibly eight, forms can be recognized by their parasitic behavior on ten selfed lines of corn.

4. Some forms are very virulent, some moderately so, while some are relatively innocuous. The most virulent form was obtained from Pennsylvania, and the weakest one from Massachusetts.

5. Some selfed lines of corn which had been resistant in the field for several years at University Farm, St. Paul, were completely susceptible to at least one of the physiologic forms.

6. Mutants occur frequently on culture media as wedge- or fan-shaped sectors. Transfers from these sectors continued to be distinct from their parents when propagated on culture media.

7. Some forms mutate readily, others do not. As a result of mutation, at least six distinct variants or mutants were obtained in a series of transfers from a single isolation. Three of these six variants originated from successive sectors.

8. The mutants differ from their parents not only in general appearance on culture media, but also in pathogenicity.

9. The mutants are as stable as forms isolated from chlamydospores obtained from smut boils. One mutant bred true for at least one and one-half years on culture media, while its pathogenic capabilities have remained constant for at least six months.

10. Distinct cultural differences were observed also between collections of *Sorosporium reilianum*.

UNIVERSITY FARM,
ST. PAUL, MINNESOTA

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PHYSIOLOGIC SPECIALIZATION OF USTILAGO NUDA AND USTILAGO TRITICI[1]

H. A. RODENHISER[2]

INTRODUCTION

Physiological specialization has long been known in the smut fungi, although the fact was not stated definitely. *Ustilago nuda* (Jens.) K. and S. and *U. tritici* (Pers.) Rost. really are physiologic forms, not good morphologic species. When *U. segetum* (Bull.) Ditt. was separated into several species, the basis for separation, at least as far as *U. nuda* and *U. tritici* are concerned, was pathogenicity, not morphology. Had the early investigators of the cereal smut fungi known what is now known about physiological specialization, it is likely that they would have considered *U. nuda* and *U. tritici* as physiologic forms of one morphologic species rather than as two morphological species. In fact, Jensen (4) did not at first elevate them to the rank of species, but considered them as varieties under the names *U. segetum* var. *tritici* and *U. segetum* var. *hordei nuda*. The morphological differences between these two fungi are scarcely pronounced enough to justify considering them as species, although it may be convenient to do so. The principal differences between them are physiologic rather than morphologic. They differ pathogenically as well as culturally, as Appel and Riehm (1) have shown.

It seemed quite probable, therefore, that there might be physiological specialization within the so-called species of loose smut fungi. This probability seemed the stronger in view of the fact that Reed (5) has demonstrated the existence of parasitic strains within *U. avenae* (Pers.) Jens. and *U. levis* (K. and S.) Magn., and Faris (2) within *U. hordei* (Pers.) K. and S., while Stakman and Christensen (7) and Tisdale and Johnston (8) have shown the same for *U. zeae* (Beck.) Ung. The writer therefore began experiments to ascertain whether there are similar strains or forms within *U. tritici* and *U. nuda*. As a preliminary to the investigation of the possible existence of pathogenic strains, an attempt was made to find out whether there were strains which could be recognized by differences in cultural characteristics on nutrient media.

[1] Published with the approval of the Director as Paper No. 645 of the Journal series of the Minnesota Agricultural Experiment Station.

[2] The writer wishes to acknowledge the helpful advice and criticism of Dr. E. C. Stakman, under whose direction the work was done.

MATERIALS AND METHODS

Collections of *U. nuda* and *U. tritici* were obtained during 1925 from widely separated localities as indicated in tables 1 and 2.[3] Pure cultures of the smuts were often obtained by transferring the chlamydospores directly to the culture medium. In other cases, chlamydospores were suspended in a one per cent solution of copper sulphate for from 10 to 27 hours. Pure cultures were then obtained by directly transferring the chlamydospores to the media by means of a platinum loop. A two per cent potato-dextrose agar proved to be the best medium for growth from which to study cultural differences of the physiologic forms of the two smuts. The pure cultures were grown for a period of two weeks on equal quantities of potato-dextrose agar. At the end of this time, triplicate series of 200 cc. Erlenmeyer flasks, containing 20 cc. of two per cent potato-dextrose agar, were inoculated with small equal portions of each culture. All of the cultures were subjected to the same environmental conditions. The agar was made up in one batch, poured and autoclaved at the same time; and the cultures were grown at room temperatures in the laboratory.

EXPERIMENTAL RESULTS

It is evident that there are distinct forms or strains, both of *U. tritici* and *U. nuda*. Cultures of *U. nuda* from six different sources and of *U. tritici* from three different sources were studied in detail. The forms can be differentiated on culture media by the following cultural characters: color, topography, character of surface, presence or absence of aerial mycelium, total amount of radial growth, and type of margin. The data on cultural characteristics are summarized in tables 1 and 2.

The cultural characteristics of six collections of *U. nuda* were distinctly different (Fig. 1). The same was true of the three cultures of *U. tritici* (Fig. 1). Furthermore, cultural differences in the various forms of *U. nuda* and *U. tritici* were as great as the cultural differences between the two species. There was, however, a distinct similarity between the culture of *U. nuda* obtained from material collected in Bulgaria and that of *U. tritici* from Egypt.

There were no apparent differences in the morphology of the various forms of the two smuts. Likewise, no microscopic differences were noted in the vegetative growths of cultures of *U. nuda* and *U. tritici* of the same age. All of the morphologic structures of *U. tritici* formed in culture and described by Sartoris (6) were noted. Chlamydospores of both *U. nuda* and *U. tritici* developed in culture.

[3] For spore material, the writer is indebted to the several people who are listed in tables 1 and 2.

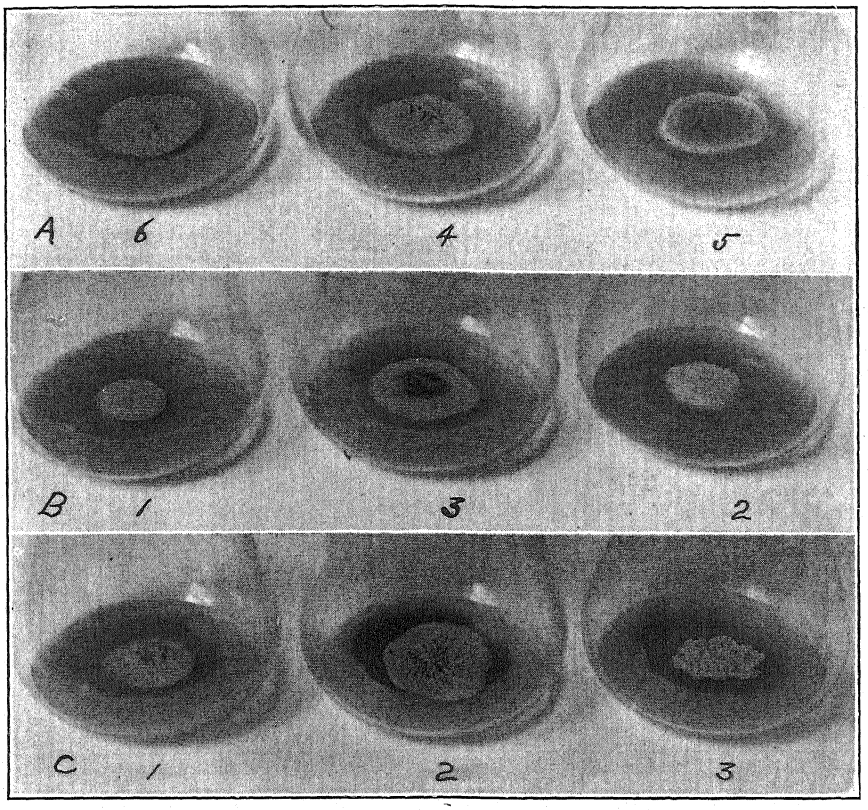

FIG. 1.—Physiologic forms of *Ustilago nuda* and *Ustilago tritici* on potato-dextrose agar, 31 days after inoculation. Series A and B, *Ustilago nuda*, forms 6, 4, 5, 1, 3 and 2. Series C, *Ustilago tritici*, forms 1, 2 and 3.

DISCUSSION

There undoubtedly is physiologic specialization in the loose smut fungi. In fact, *U. nuda* and *U. tritici* are themselves nothing more nor less than physiologic forms. The chlamydospores of the two are almost exactly alike; neither produces sporidia, and the effect on the host plants is essentially the same. The principal difference between them is that *U. nuda* infects barley, whereas *U. tritici* infects wheat. It is true that there are some differences on culture media, but the facts brought out in this paper show clearly that too much significance can not be attached to these differences. If we consider *U. nuda* and *U. tritici* as species, there are at least several physiologic forms within each species. It is significant that some of the forms of *U. tritici* resemble some of *U. nuda* more closely than they do certain forms

TABLE 1.—*Cultural characteristics of physiologic forms of Ustilago nuda on potato-dextrose agar*

Source of collection	Form	Age in days	Size in mm.	Color	Topography	Surface	Aerial mycelium[a]	Margin
Minnesota Writer	1	20	9	Dirty white	Center capitate; ray appressed	Center coarse, felt-like; ray china-like	+=	Almost entire; myceloid
		31	19	Center becoming brown, grading to gray white	Center convex to pulvinate; ray appressed	do	+	do
Manitoba Writer	2	20	13	Creamy white	Center pulvinate; ray appressed	Long fine felt-like strands	+	Entire; myceloid
		31	23	do	do	Velvet to felt-like	+	do
California F. W. Briggs	3	20	15	Center white, grading to cream	Umbonate and striated	Center of long strands; coarse, felt-like	+-	Almost entire; myceloid
		31	28	Center peppered with deep brown spots, grading to cream	Umbonate; covered with numerous small tufts; margin appressed	Moist to velvety	+	do
Bulgaria A. Atanasoff	4	20	14	Center cream, grading to white	Raised to convex; smooth center, grading to coarse striations in the ray	Moist to waxy	+=	Slightly undulating
		31	27	Center tan, grading to white	Raised, ridged and striated counter-clockwise	Waxy to china-like	+=	Slightly undulating; very slightly myceloid
Belgium E. Marchal	5	20	21	Center cream, grading to dirty white	Convex to umbonate	Center of long strands; coarse, felt-like	+-	Almost entire; slightly myceloid
		31	28	Center peppered with black tufts, grading to dirty tan	do	Moist to velvety	0	Almost entire; not myceloid

TABLE 1.—(*Continued*)

Source of collection	Form	Age in days	Size in mm.	Color	Topography	Surface	Aerial mycelium[a]	Margin
Hungary B. Husz		20	21	Center tan, grading to white and almost hyaline margin	Radially striated; umbonate	Waxy to china-like	+ =	Undulate; myceloid
	6	31	30	do	Radially striated; umbonate, with fine rope-like strands	do	0	Undulate; not myceloid

[a] 0 indicates no aerial mycelium.
+ indicates aerial mycelium abundant.
+ – indicates slight amount of aerial mycelium.
+ = indicates trace of aerial mycelium.

TABLE. 2.—*Cultural characteristics of physiologic forms of Ustilago tritici on potato-dextrose agar*

Source of collection	Form	Age in days	Size in mm.	Color	Topography	Surface	Aerial mycelium[a]	Margin
Minnesota Writer	1	20	14	Dull white	Flat and finely striated	Waxy	0	Undulate to lobate; myceloid
		31	34	Cream center grading to dull white	Flat and finely striated, becoming deeply ridged	do	+ =	Slightly undulate; myceloid
Egypt T. Fahmy	2	20	23	Cream center grading to dull white	Umbonate and coarsely striated	Moist to china-like	+ =	Undulate; myceloid
		31	31	Tan center grading to cream	Raised to convex counterclockwise striations	do	+ =	Slightly undulate; no longer myceloid
New South Wales R. J. Noble	3	20	20	Cream to faint pink	Ribbon-like heaps	Dull china-like	0	Lobate to lacerate
		31	25	do	Ribbon-like heaps raised to 7–8 mm.	do	0	do

[a] 0 indicates no aerial mycelium.
+ = indicates trace of aerial mycelium.

of *U. tritici*. This would seem to indicate that there probably are many forms and that the specialization is not necessarily restricted to wheat or barley. In fact, Humphrey and Tapke (3) have already shown that *U. tritici* can infect rye, and the writer considers it quite probable that some of the forms of *U. tritici* probably will be able to infect barley and vice versa.

While the differences in forms which the writer has demonstrated are cultural and not pathogenic, they are very pronounced and permanent. In fact, the cultural differences between forms of *U. tritici* are sometimes more pronounced than those between so-called *U. tritici* and *U. nuda*. It has been shown clearly that these forms are not mere variants but are constant. Naturally, environmental conditions have a profound effect on the development of the different forms, but under the same conditions they can always be recognized.

Whether these forms will be different pathogenically remains to be seen. It would be rather surprising if they are not. Experiments are now under way to determine this fact.

UNIVERSITY FARM,
ST. PAUL, MINN.

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PHYTOPATHOLOGICAL NOTES

Powdery Mildew of Muskmelons in the Imperial Valley of California in 1925.—A large and increasing acreage of early muskmelons (usually known in the West and South as cantaloupes) is grown in the Imperial Valley of California. For 1925 it is estimated that there were 30,000 acres, from which 5,100,000 crates, or 15,000 cars, were shipped to market. The climate of the Valley is extremely arid, with an average annual rainfall of about three inches and a very high percentage of sunshine. Before 1925 there had been no complaint of injury to melon foliage by the numerous fungi and bacteria which attack the foliage in moister climates, and apparently no record of the occurrence of a single organism parasitic on the leaves.

About May 10, 1925, when harvesting was just beginning, local growers and shippers became disturbed over the appearance of powdery mildew, which had apparently never before been observed on muskmelon in the Valley. At that time there were in several of the earlier fields small isolated spots, one to three feet in diameter, where practically all the leaves were dead and drooping, with leaves, petioles, and stems literally covered with the characteristic white to grey powdery growth of mildew. For some distance from the spots isolated, leaves showed young, actively growing mildew. The disease spread rapidly, and by June 6 a high percentage of the older leaves in many of the earlier fields had been killed, giving to the fields a dead-brown appearance. Invariably there was no foliage injury and little or no mildew to be found until melons began to ripen, but in practically every field in the Valley mildew appeared at about that stage and developed rapidly thereafter. The first or main set of melons was not materially reduced in quantity, although it is probable that quality was affected, particularly in the case of later pickings, when many of the older leaves had been killed. By the time the first set of fruits had been harvested the foliage was reduced so much that later set fruits failed to mature. The first set normally makes up a large percentage of the crop in Imperial Valley, and it is generally agreed that the reduction in yield amounted to only about 15 per cent for the whole Valley. It is possible that reduction in quality was of fully as great importance, although neither data nor estimates are obtainable.

Several varieties of muskmelons, including the Honey Dew and other members of the Casaba group, are grown in the Valley, and all were injured by mildew. Various fungicides in both dust and liquid form were applied by the county agent and by several growers, but little or no control of the

mildew was obtained. Failure of the fungicides may have resulted largely or wholly from faulty application, as the mildew was well established before the first applications were made and the machines were not of the most efficient types. Dusting with sulphur was out of the question, as either alone or mixed with hydrated lime it burned the foliage so severely that it was more destructive than the mildew.

At first it seemed that the outbreak might be the result of a rather unusual season, as favorable weather in March and April brought the crop to harvest ten days earlier than ever before, and there were several cool, cloudy days, rather unusual for that season of the year, at the time mildew was first observed. During June, however, sunny weather, high temperature, and low humidity in fully normal degree were the rule. Mildew spread rapidly from the time of its appearance until the end of the harvest in mid-July, with no indications of being influenced by changes in weather. It is probable, therefore, that the fungus was introduced in 1925. The fact that Imperial Valley is surrounded by mountains and desert, with specialized desert vegetation, would account for the absence of the fungus previous to this time.

Perithecia of the fungus have not been found. Consequently it has been impossible definitely to classify it, although *Erysiphe cichoracearum* DeC. usually is found on cucurbits. Failure to find perithecia raises the question of the ability of the parasite to live over to the next crop. In addition to the possibility that perithecia occur rarely, the fungus may be able to persist in vegetative form. Mildew occurred on volunteer melon plants throughout the summer and fall of 1925. Frosts during December and January usually kill all unprotected plants, but on December 30, under the protection of a hedgerow, volunteer plants were found with a few living leaves covered with mildew. Planting of the next crop began early in December under oiled-paper frost protectors, and on December 30 the seedlings showed one or two leaves each, although no mildew was found on them.

The probability of powdery mildew attacking the Imperial Valley melon crop in destructive form each season henceforth is strongly suggested. It is possible, however, that the outbreak of 1925 was of sporadic nature, resulting from some unusual factor or combination of factors, and that nothing similar will occur again for many years.[1]—IVAN C. JAGGER, Office of Vegetable and Forage Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[1] In 1926 mildew appeared earlier in the season and was decidedly more destructive, reducing the yield for Imperial Valley from an average of 160 crates per acre to an average of 110 crates.

Black End of Apple.—Observations made on two apple trees at Grady, Montgomery County, Alabama, August 28, 1925, showed a fairly high percentage of the fruits to be affected with the malady described by Barss[1] as "black end." This trouble, though apparently not uncommon on apples and pears in the hot dry sections of Oregon and Washington, impressed the writer as being rather unusual in the Southern States.

The malady is characterized, as shown in figure 1, by a very black discoloration of the skin surrounding the calyx of the fruit. The affected region is circular and measures about 2.5 cm. in diameter. There is a definite margin separating the blackened region from the healthy skin, and the affected area is usually slightly depressed. The flesh is involved only to a very slight extent, but the affected skin and tissue immediately beneath become dry and leathery. As subsequent growth of the fruit occurs, a crack is formed along the margin of the affected area, separating the normal from the abnormal tissue.

Upon first inspection one is liable to confuse black end with arsenical spray injury, due to the similarity of the two disorders. This did not occur in the case under consideration because no spray had been applied. The

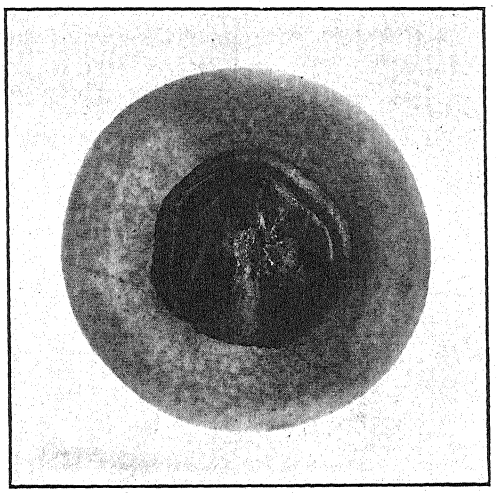

FIG. 1.—A typical case of black end on an apple. x 1.

trouble seemed to be unquestionably the result of the hot dry season, the drought being the most severe in many years. Records show that there was very little rainfall in the locality from February to October. Light rains fell April 4, June 16 and July 18. From this date until October there was

[1] BARSS, H. P. Physiological disorders of developing fruits. Third Crop Pest and Hort. Rept. Ore. Agr. Col. Exp. Sta., 1915–1920. Pp. 159–166. 1921.

no appreciable rainfall. The soil in the orchard was red clay. This no doubt made the drought injury more severe.—S. A. WINGARD, Virginia Agricultural Experiment Station, Blacksburg, Va.

Crown Wart of Alfalfa in the South.—In a recent number of PHYTOPATHOLOGY (Vol. 15, p. 807) the writer reported having found the crown wart of alfalfa near Madison, Indiana. This was thought to be the first report of the occurrence of this disease east of the Rocky Mountains. Further evidence of the distribution of the disease in Eastern United States was obtained in May, 1926, while inspecting alfalfa fields in Mississippi and Alabama. The disease was found in two fields near Muldon and in two fields near Columbus, Mississippi. Later the disease was found both at Uniontown and at Selma, Alabama. Altogether it was found in seven different fields. It is probable that further search would have revealed its presence in still other fields. In most cases a very high percentage of the plants were affected. In many cases the diseased buds either failed to produce stems, or the stems were stunted and sometimes died after they were partly grown. However, in most of the fields the plants were sufficiently thick so that the loss of even a considerable number of stems would not ordinarily be noticed. There was little indication that entire plants were dying, although it was observed that portions of the crowns of some plants apparently were killed by the disease.

Two fields at Uniontown, Alabama, about a mile apart, which were sown at the same time with the same seed had a high percentage of the affected plants. Another field sown at a different date and with other seed and separated from one of the two diseased fields only by a small drainage ditch was apparently free of disease. This points to the possibility that the disease was seedborne. Although the seed dealer is known, no positive proof as to the source of the seed could be obtained.—J. L. WEIMER, Office of Vegetable and Forage Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Kansas Agricultural Experiment Station.

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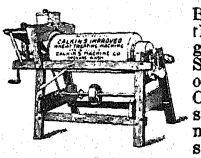

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